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Pharmacokinetic and pharmacodynamic interactions of tolfenamic acid and marbofloxacin in goats

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Abstract

Pharmacokinetic and pharmacodynamic properties in goats of the non-steroidal anti-inflammatory drug tolfenamic acid (TA), administered both alone and in combination with the fluoroquinolone marbofloxacin (MB), were established in a tissue cage model of acute inflammation. Both drugs were injected intramuscularly at a dose rate of 2 mg kg^{-1}. After administration of TA alone and TA + MB pharmacokinetic parameters of TA (mean values) were C_{max} = 1.635 and 1.125 μg ml^{-1}, AUC = 6.451 and 3.967 μg h ml^{-1}, $t_{1/2}K_{10}$ = 2.618 and 2.291 h, Vdarea/F = 1.390 and 1.725 L kg^{-1}, and ClB/F = 0.386 and 0.552 L kg^{-1} h^{-1}, respectively. These differences were not statistically significant. Tolfenamic acid inhibited prostaglandin (PG)E_2 synthesis in vivo in inflammatory exudate by 53–86% for up to 48 h after both TA treatments. Inhibition of synthesis of serum thromboxane (Tx)B_2 ex vivo ranged from 16% to 66% up to 12 h after both TA and TA + MB, with no significant differences between the two treatments.

From the pharmacokinetic and eicosanoid inhibition data for TA, pharmacodynamic parameters after dosing with TA alone for serum TxB_2 and exudate PGE_2 expressing efficacy (E_{max} = 69.4 and 89.7%), potency (IC_{50} = 0.717 and 0.073 μg ml^{-1}), sensitivity (N = 3.413 and 1.180) and equilibration time ($t_{1/2}K_{e0}$ = 0.702 and 16.52 h), respectively, were determined by PK–PD modeling using an effect compartment model. In this model TA was a preferential inhibitor of COX-2 (COX-1:COX-2 IC_{50} ratio = 12:1). Tolfenamic acid, both alone and co-administered with MB, did not affect leucocyte numbers in exudate, transudate or blood. Compared to placebo significant attenuation of skin temperature rise over inflamed tissue cages was obtained after administration of TA and TA + MB with no significant differences between the two treatments. Marbofloxacin alone did not significantly affect serum TxB_2 and exudate PGE_2 concentrations or rise in skin temperature over exudate tissue cages. These data provide a basis for the rational use of TA in combination with MB in goat medicine.

Keywords: Goat; Tolfenamic acid; Marbofloxacin; Pharmacokinetics; Pharmacodynamics; Thromboxane B_2; Prostaglandin E_2; PK–PD modeling

1. Introduction

Tolfenamic acid (TA) [*N*-(2-methyl-3-chlorophenyl) anthranilic acid] is a non-steroidal anti-inflammatory drug (NSAID) of the fenamate sub-group. In common with other NSAIDs, the principal mechanism of action for both pharmacological and toxic effects is believed to be inhibition of cyclo-oxygenase (COX) (Linden et al., 1975, 1976; McKellar et al., 1994a; Landoni et al., 1995b, 1996a,b; Lees et al., 1998). This enzyme catalyses the conversion of arachidonic acid to pro-inflammatory prostaglandins (Vane, 1971). However, TA exerts other actions at molecular and tissue levels; for example, it possesses anti-oxidant properties (Kourounakis et al.,

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2000) and inhibits some actions of prostaglandins as well as their synthesis (Li et al., 1998). Moreover, in vitro and ex vivo studies in man have indicated that it produces dual inhibition of COX and 5-lipoxygenase (5-LO) (Alanko et al., 1989; Moilanen et al., 1989). However, studies demonstrating no effect of therapeutic dose rates of TA on 5-LO in vivo have been reported in man and cattle (Proudman and McMillan, 1991; Landoni et al., 1995b, 1996a).

Tolfenamic acid is a potent inhibitor of ex vivo serum thromboxane (Tx)B_2 synthesis (formed when blood is allowed to clot under controlled conditions) in dogs and calves (McKellar et al., 1991, 1994a; Landoni et al., 1996a; Lees et al., 1998; Sidhu et al., 2005). Moreover, prostaglandin (PG)E_2 generation in calf inflammatory exudate was reduced to a similar extent by TA (2 mg kg^{-1}) and flunixin (2.2 mg kg^{-1}), another NSAID of the fenamate class (Landoni et al., 1995a,b, 1996a). The latter authors used a PK–PD modeling approach, based on the sigmoid E_{max} equation and an effect compartment model, to determine a slightly greater IC_{50} of TA for inhibition of serum TxB_2 synthesis than for blockade of exudate PGE_2 synthesis in calves (ratio = 1.78:1.00). Sidhu et al. (2005) reported a slightly lower ratio of 1.37:1.00 also in calves in a similar model. These data suggest that in this species TA may be classified as an essentially non-selective inhibitor of the COX isoforms, COX-1 and COX-2, although Lees (2003) reported that for 95% inhibition of PGE_2 in exudate there was only 59% inhibition of TxB_2 in serum.

Tolfenamic acid has been used extensively in humans and in dog, cat, calf and pig medicine for its anti-inflammatory, analgesic and antipyretic properties. For example, TA is used clinically as an anti-inflammatory and analgesic agent for the therapy of locomoter diseases in the dog (Robertson and Taylor, 2004) and febrile syndromes and post-operative pain in cats (Slingsby and Waterman-Pearson, 2000). Its anti-inflammatory and anti-endotoxaemic properties may also be used to enhance the rate of recovery in combination with antimicrobial drugs in acute mastitis in cattle, in pneumonia and other viral and bacterial respiratory diseases in calves and in pigs for treatment of the metritis–mastitis–agalactia syndrome. For example, TA has been used in combination with oxytetracycline, in the therapy of pneumonia in cattle, when it improved clinical resolution (Deleforge et al., 1994).

Although TA itself is not known to possess antimicrobial actions when administered at clinical dose rates in species of veterinary interest, apparent antibacterial actions have been reported in humans (Kruszewska et al., 2002). Tolfenamic acid and the antimicrobial fluoroquinolone drug marbofloxacin (MB) are licensed in the European community and marketed by the same manufacturer, for the therapy of calf respiratory disease. Therefore, it is likely that they will be used frequently in combination and this consideration motivated the current investigation. Moreover, there are reports of anti-inflammatory properties of fluoroquinolones (Hoeneb et al., 2000; Davies et al., 2004; Hostutler et al., 2004) so that in combination with NSAIDs they may exert additive or complementary anti-inflammatory effects.

The goat is a minor species for which very few drugs are licensed for clinical use. However, as in calves, pneumonia in the goat is a condition which requires therapy and antimicrobial drugs are likely to be used both alone and in combination with NSAIDs. However, there is a dearth of literature in the goat on the pharmacokinetics and pharmacodynamics of drugs of both classes, yet such data are essential as a basis for designing dosage schedules for clinical use. Moreover, from the limited published literature in this field, it is clear that data cannot be transposed from species such as the calf to the goat (Landoni and Lees, 1995a; Arifah et al., 2003). The present investigation was therefore undertaken with the following objectives: (1) to establish the serum concentration-time profile and pharmacokinetic parameters for TA after intramuscular administration in goats at the dose rate recommended for calves; (2) to determine pharmacokinetic parameters for TA after co-administration with MB also injected intramuscularly at the dose rate recommended for calves to establish any interaction between the drugs; (3) to determine in a tissue cage model the rate and extent of penetration of TA into inflamed (exudate) and non-inflamed (transudate) cage fluids after administration of TA alone and in combination with MB; (4) to investigate the degree and time course of inhibition of the COX isoenzymes, COX-1 and COX-2, in order to derive pharmacodynamic parameters for TA; (5) to establish the degree and time course of attenuation by TA and TA + MB of a clinical surrogate response, rise in skin temperature, over the surface of inflamed cages; and (6) to investigate the effects of TA alone and TA + MB on the recruitment of leucocytes into inflammatory exudate.

2. Materials and methods

2.1. Animals and surgical procedures

The study was carried out in eight healthy female, non-lactating goats of the Saanen breed of weight 50–85 kg and age 1.0–2.5 years. They were maintained in a ventilated barn in individual pens separated by wire mesh barriers to maintain social contact. Each animal had free access to hay and water, and were given daily 1 kg of pelleted small ruminant diet. Four cylindrical tissue cages prepared from silicone rubber tubing were

inserted subcutaneously in each animal, two on each side of the neck approximately equidistant from the jugular vein and spinal cord as previously described (Sidhu et al., 2003). Animals were allowed to recover from surgery for 7 weeks to permit wound healing and the growth of granulation tissue into and around the cages. The study was approved by the Royal Veterinary College Ethics and Welfare Committee.

2.2. Experimental design, sampling procedures and model of inflammation

Tolfenamic acid (Tolfedine 4%, Vetoquinol Ltd., UK) and MB (Marbocyl 10%, Vetoquinol Ltd., France) were provided by the manufacturer. A four-period cross-over design was used, such that each goat received TA, MB, TA + MB and placebo (PL) treatments in a randomized sequence. All injections were made at zero time into the thigh muscles. The dose rate was 2 mg kg^{-1} for each drug, selected on the bases that this is the recommended dose rate for use in calves and there are no previous publications with either drug in the goat. Each animal received two injections in each of the four cross-over periods and these were administered into opposite thigh muscles. The PL injection was sterile normal saline, administered in a dose volume equivalent to 2 mg kg^{-1} TA. An interval of 14 days was allowed between each period.

Blood samples (10 ml) were collected for the measurement of serum TA concentration before and at times of 5, 10, 15, 20, 30 and 45 min and 1, 1.5, 2, 3, 4, 6, 9, 12, 24, 30, 36 and 48 h after drug administration. Samples were placed, protected from light, at room temperature for 30 min, transferred to ice for 30–60 min and then centrifuged at 4 °C to obtain serum. Further blood samples (2.5 ml) were collected in KEDTA for the determination of blood total leucocyte count. For measurement of serum TxB_2 concentration, 2.0 ml blood samples were collected at times of 1, 3, 6, 9, 12, 24, 30, 36, 48 and 72 h.

At time 0 in each period, an intra-caveal injection of 0.5 ml of 1% sterile carrageenan solution (Viscarin GP 109, Marine Colloids, Springfield, USA) was administered into one tissue cage. This cage was used to collect exudate (1.2–1.5 ml) samples at pre-determined times (1, 3, 6, 9, 12, 24, 30, 36 and 48 h). A non-injected tissue cage was used to collect non-inflamed tissue cage fluid (transudate, 1.2–1.5 ml) at the same times. For each period of the cross-over, exudate was generated in a cage which had not been used to generate exudate in a previous period.

2.3. Measurements and analytical methods

Concentrations of tolfenamic acid in serum, exudate and transudate were determined by an HPLC method with UV detection. A volume of 0.5 ml serum or 100 μl of exudate or transudate was acidified with 1.0 ml citrate phosphate buffer (pH 3.0). Five ml chloroform containing 0.5 μg ml^{-1} mefenamic acid (internal standard) was added and the contents mixed for 15 min. After centrifugation at 2000*g* and 20 °C for 20 min, 4 ml of the organic phase was collected and evaporated to dryness under nitrogen at 50 °C. The residue was reconstituted in 150 μl methanol and dissolved by vortexing for 15 s. The HPLC system comprised a system controller (Waters 600 E, Milford, MA, USA), a tunable absorbance detector (Waters 484), a Shimadzu autoinjector (SIL-6A, Kyoto, Japan) and integrator (Waters 746). The pre-column and column used were: Lichrosorb RPC_{18}5μm and Lichrosorb RPC_{18}7 μm (250 × 4 mm) with UV detection (342 nm). The mobile phase was a mixture of 80% methanol:20% water, containing 0.001% perchloric acid and degassed with helium. The injection volume was 30 μl and the flow rate was 1 ml min^{-1}. The elution times for TA and internal standard were approximately 9 and 11 min, respectively. Concentrations of TA were calculated using ratios of peak area. Reagents were supplied by Sigma–Aldrich Chemicals (Poole, Dorset, UK).

The LOQ for TA in serum, exudate and transudate was 0.05 μg ml^{-1}. The concentration/peak area relationship was linear ($r^2 > 0.999$) over the concentration range 0.05–10.0 μg ml^{-1}. There was no interference from MB in the TA assay. The repeatability and reproducibility of the method were evaluated using intra-assay and inter-assay values from spiked concentrations and coefficients of variation (CV%) for intra-assay and inter-assay were 6.75% and 9.56%, respectively, at a concentration of 4 μg ml^{-1} and 9.22% and 10.54% at a concentration of 0.5 μg ml^{-1}. Percentage accuracies and precision were 90.5% and 5.5% in the absence of MB and 90.3% and 7.3% in the presence of MB.

For the determination of serum TxB_2 concentration, blood was allowed to clot immediately after collection for 60 min in glass tubes in a water bath at 37 °C. Samples were then placed on ice prior to centrifugation (4 °C, 2000*g*, 10 min) to separate serum. Exudate and transudate were also centrifuged (4 °C, 2000*g*, 10 min) to remove cells. Serum, exudate and transduate samples were stored at −20 °C prior to analyses. Concentrations of serum TxB_2 and exudate PGE_2 were determined using radioimmunoassay (RIA) methods previously described by Higgins and Lees (1984b). For PGE_2 LOD was 0.01 ± 0.01 ng ml^{-1}, the quantification range was 0.05–5.0 ng ml^{-1} and percentage B/F range was 97.1 ± 2.0% to 4.1 ± 1.5%. For TxB_2 LOD was 0.01 ± 0.01 ng ml^{-1}, the quantification range was 0.05–5.0 ng ml^{-1} and percentage B/F range was 96.5 ± 1.9% to 1.6 ± 0.8%.

Skin temperature (°C) over the tissue cages was monitored using an Horiba IT-330 Infrared thermometer (Horiba Instruments, Tokyo, Japan). Total leucocyte counts in serum, exudate and transudate were measured

using a Coulter Counter (Model ZM-Coulter Electronics Ltd., UK) within 2 h of sample collection.

2.4. Pharmacokinetic and PK–PD analyses

Serum, exudate and transudate TA concentration-time data for individual goats were analysed using WINNOLIN compartmental programs by non-linear least squares regression analysis (Pharsight Corporation, Mountain View, CA, USA). Schwarz criteria and minimum Akaike Information Criteria Estimates were applied to discriminate the best fitting model and improved fit of data was achieved by re-weighting.

PK–PD modeling for inhibition of serum TxB_2 and exudate PGE_2 was based on extended non-linear least-squares regression analysis, using the MK model program (Biosoft, Cambridge, UK). Because of the hysteresis present in the relationship between NSAID serum concentration and measured effect, a mamillary compartment model was applied (Landoni and Lees, 1995a,b). In this model, the biophase is assumed to receive a negligible amount of drug and to have a negligible volume (Sheiner et al., 1979; Holford and Sheiner, 1981, 1982). To relate serum concentration of TA to observed effects, a Hill plot was applied:

$$E = E_0 \pm \frac{E_{max} \times Ce^N}{IC_{50}^N + Ce^N},$$

where E_0 is the control value, E_{max} the maximal effect, Ce the apparent drug concentration in the effect compartment, IC_{50} the plasma drug concentration producing 50% of E_{max} and N the slope of the log concentration/effect relationship. Initial estimates of E_{max} and IC_{50} were calculated graphically from simulated concentrations.

From previous studies, it is assumed that inhibition of synthesis of serum TxB_2 in clotting blood ex vivo is due solely to an action on COX-1 (Warner et al., 1999). It is also *tentatively* assumed that inhibition of exudate PGE_2 in vivo is due to an action on COX-2, although some contribution from COX-1 inhibition cannot be excluded.

2.5. Statistical analyses

Differences between animals, times, sequence and cross-over periods and their associated two factor interactions were analysed by ANOVA using a MINITAB Software program (Version 10.1, Minitab Inc., State College, PA, USA). For pharmacodynamic measurements the significance of differences at each time point between treatment groups and between pre-treatment and post-treatment values was further investigated using Fisher's multiple comparisons test. For pharmacokinetic parameters the significance of differences was assessed using the Mann–Whitney test. All tests of significance were carried out at a 5% level.

3. Results

3.1. Pharmacokinetics

3.1.1. Serum TA concentration

Mean (±SD) concentrations of TA in serum after the administration of TA and TA + MB intramuscularly are presented in Fig. 1. The drug was present in detectable concentrations at all sampling times between 5 min and 12 h. By 5 min the mean concentrations were 1.544 and 0.931 μg ml^{-1} after TA and TA + MB, respectively. Maximum drug concentrations in serum were achieved at 0.193 and 0.261 h after TA and TA + MB, respectively, and corresponding C_{max} values were 1.635 and 1.125 μg ml^{-1} (Table 1). Concentrations were higher in goats receiving TA alone (AUC = 6.451 versus 3.967 μg h ml^{-1}) but the difference was not statistically significant.

After both TA and TA + MB administrations, serum concentration-time curves were best fitted to an absorption with one-compartment elimination model in 13 of 16 instances and a two-compartment elimination model in three instances. Derived pharmacokinetic parameters

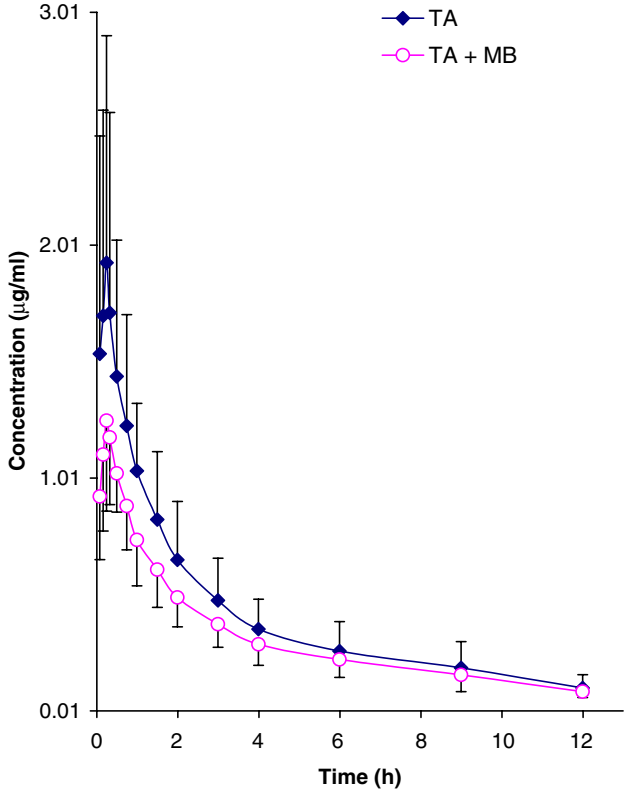

Fig. 1. Arithmetic plot of plasma concentration of TA versus time after administration of TA and TA + MB. Each point is the mean ± SD for eight goats.

Table 1
Pharmacokinetic parameters of TA in serum after administration of TA and TA + MB (mean and SD, $n = 8$)

Parameter	TA		TA + MB	
	Mean	SD	Mean	SD
K_{01} (h^{-1})	25.74	10.07	17.36	6.19
K_{10} (h^{-1})	0.276	0.057	0.326	0.084
$t_{1/2}K_{01}$ (h)	0.030	0.001	0.047	0.028
$t_{1/2}K_{10}$ (h)	2.618	0.622	2.291	0.679
C_{max} (μg ml^{-1})	1.635	0.848	1.125	0.283
T_{max} (h)	0.193	0.057	0.261	0.113
AUC (μg h ml^{-1})	6.45	3.34	3.97	1.22
Vdarea/F (L kg^{-1})	1.390	0.537	1.725	0.396
$MRT_{(0\text{-last})}$ (h)	5.06	1.47	5.59	1.30
CIB/F (L kg^{-1} h^{-1})	0.386	0.198	0.552	0.198

Pharmacokinetic parameters were calculated using a one compartment model with first order input and output: K_{01}, absorption rate constant; K_{10}, elimination rate constant; $t_{1/2}K_{01}$, absorption half-life; $t_{1/2}K_{10}$, elimination half-life; C_{max}, maximum concentration in plasma; T_{max}, time of maximum plasma concentration; AUC, area under plasma concentration-time curve; Vdarea/F, ratio of volume of distribution to bioavailability; MRT, mean residence time to last sampling time; CIB/F, ratio of clearance to bioavailability. MRT was calculated by non-compartmental modeling. There were no statistically significant differences between TA and TA + MB treatments.

were based on a one-compartment model in all animals. Clearance, scaled by bioavailability, was numerically greater after TA + MB dosing compared to TA alone (0.552 versus 0.386 L kg^{-1} h^{-1}) but the difference was not statistically significant (Table 1). Mean residence time and terminal half-life were similar after the two treatments.

3.1.2. Exudate and transudate TA concentrations

Tolfenamic acid was present in exudate and transudate in quantifiable concentrations in every instance 3 h after administration (Fig. 2), but concentrations in both fluids were initially lower than in serum. Exudate TA concentration exceeded that in serum by 6 h and drug was detectable in exudate up to 30 h. Transudate concentrations were numerically somewhat lower than in exudate, as reflected in C_{max} and AUC values but the differences were not statistically significant (Table 2). Exudate and transudate data fitted a one-compartment model with lag phase and first order input and output. Tolfenamic acid concentrations in both fluids peaked between 6.36 and 9.28 h. Slow penetration of TA into and prolonged persistence in both exudate and transudate was indicated by the values of lag time and mean residence time, respectively. Similar findings were obtained for the combined administration of TA and MB.

The AUC ratios (converted to percentages, serum = 100%) for exudate and transudate to serum were 117 ± 42 and 74 ± 42%, respectively, in TA treated goats. Similar AUC ratios for exudate:serum of 118 ± 68% and for transudate: serum of 83 ± 246% were obtained in TA + MB treated calves.

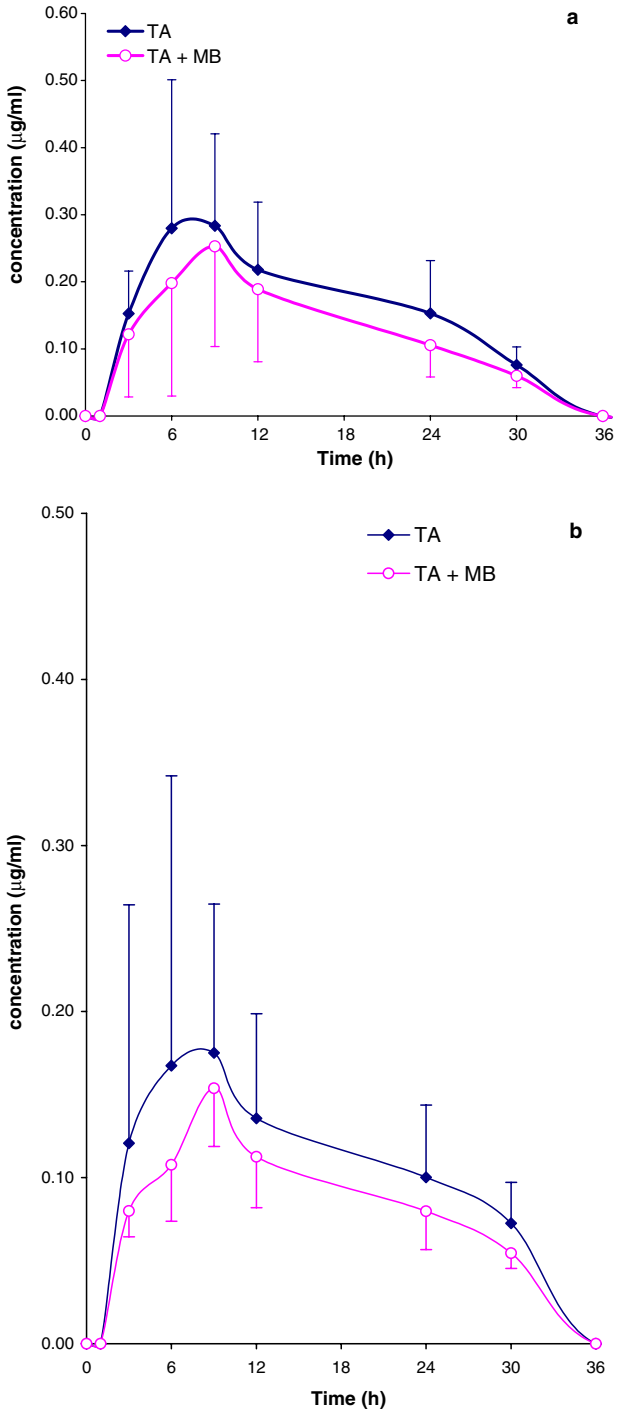

Fig. 2. Arithmetic plot of (a) exudate concentration and (b) transudate concentration versus time of TA after administration of TA and TA + MB. Each point is the mean ± SD for eight goats.

3.2. Pharmacodynamics

3.2.1. Serum TxB_2

In goats receiving PL only, ex vivo serum TxB_2 production remained relatively constant between 1 and 48 h and differences were not significant compared to 0 h values (Fig. 3). Tolfenamic acid inhibited TxB_2 synthesis

Table 2
Pharmacokinetic parameters of TA in exudate and transudate after administration of TA and TA + MB (mean and SD, $n = 7$)

	Exudate				Transudate			
	TA		TA + MB		TA		TA + MB	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
AUC (μg h ml^{-1})	6.71	3.00	4.56	2.52	4.08	1.87	2.95	0.82
C_{max} (μg ml^{-1})	0.328	0.226	0.258	0.170	0.219	0.198	0.153	0.028
T_{max} (h)	6.36	1.83	9.00*	2.35	9.28	3.05	7.66	1.50
T_{lag} (h)	3.20	1.92	2.95	1.67	2.25	1.81	3.23	2.29
$MRT_{(0\text{-last})}$ (h)	15.75	1.27	14.59	1.02	16.59	2.94	15.20	1.41

Pharmacokinetic parameters: T_{lag}, lag time. For other parameters, see Table 1. Parameters were calculated using a one compartment model with first order input and output. MRT was calculated by non-compartmental modeling. $^{*}P < 0.05$ for comparison of TA and TA + MB. There were no statistically significant differences between exudate and transudate for either TA or TA + MB treatment. The only significant difference between TA and TA + MB was for exudate T_{max}.

($P < 0.05$) at all sampling times between 1 and 12 h with both administrations (TA and TA + MB) compared both with the PL treatment and control (zero time) values (Fig. 3). Inhibition was maximal (62–66%) at 1 h. No significant difference in the degree of TxB_2 inhibition was obtained between TA and TA + MB treatments. Thromboxane B_2 concentration had returned to control by 24 h after administration with both TA and TA + MB. With MB alone there was no effect on serum TxB_2 concentration (Fig. 3).

3.2.2. Exudate PGE_2

Prostaglandin E_2 concentrations in exudate were low but quantifiable at early sampling times (1 and 3 h) in goats receiving PL (Fig. 4). Concentrations were maximal (40.9 ng ml^{-1}) at 30 h. The PGE_2 concentration change with time was highly significant ($P < 0.001$). With both TA and TA + MB administrations, exudate PGE_2 concentrations were inhibited significantly at all times between 3 and 48 h compared with PL-treated animals ($P < 0.05$). The mean percentage inhibition of PGE_2 synthesis was in the range of 53–86% between 3 and 48 h after administration of TA or TA + MB compared to PL With MB administration, PGE_2 concentrations were not altered at any sampling time.

3.2.3. Skin temperature over tissue cages

Increased skin temperature over tissue cages was recorded at all sampling times after intra-caveal carrageenan injection in goats receiving PL (Table 3). However, the peak rise in temperature (2.05 °C) at 24 h was relatively small. Compared to baseline temperature, significant increases occurred at 9, 12, 24, 30, 36 and 48 h after the injection ($P < 0.05$).

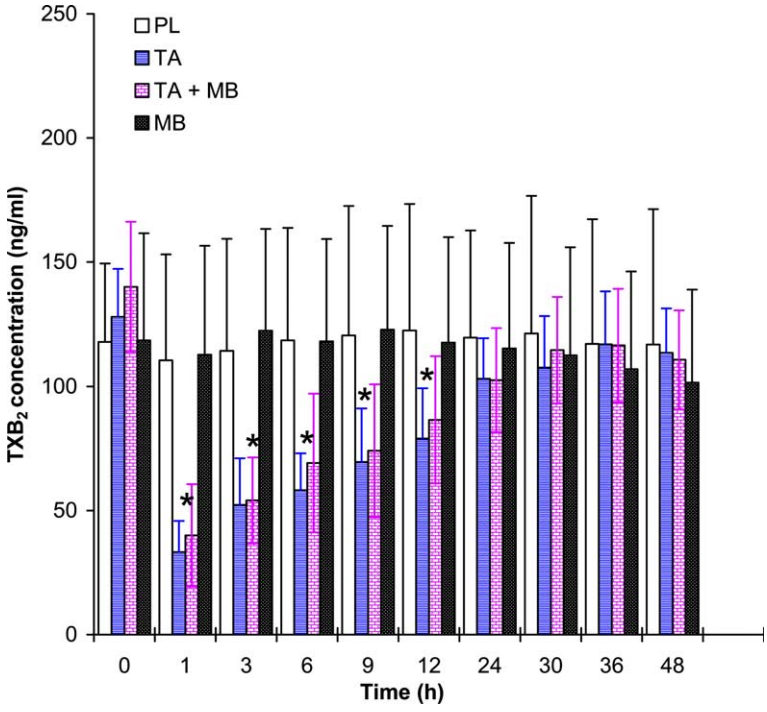

Fig. 3. Time course of TxB_2 production in serum after administration of placebo, TA, TA + MB and MB. Values are means ± SD ($n = 8$). $^{*}P < 0.05$ compared with placebo treatment (refers to both TA and TA + MB).

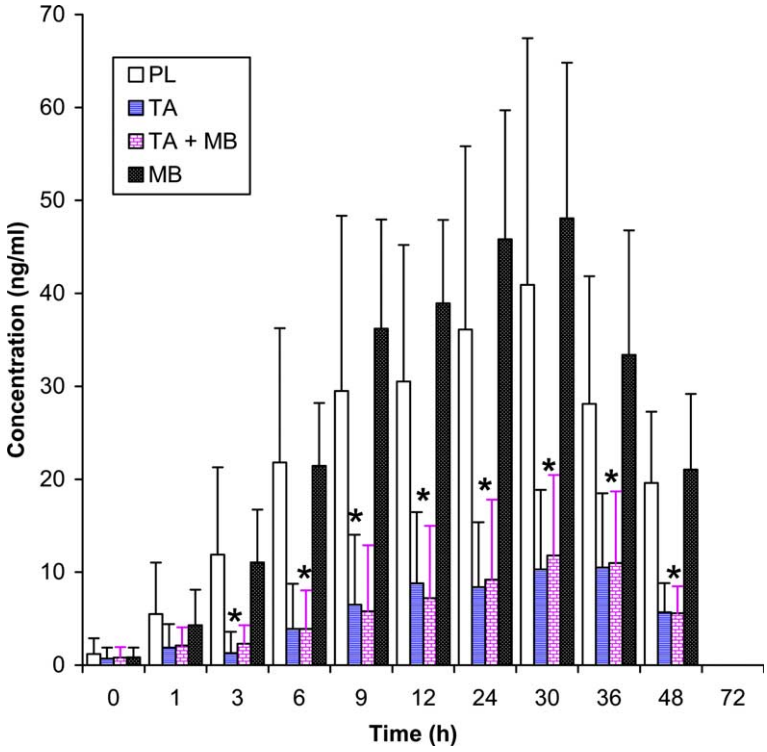

Fig. 4. Time course of PGE_2 production in exudate after administration of placebo, TA, TA + MB and MB. Values are means ± SD ($n = 8$). *$P < 0.05$ compared with PL treatment (refers to TA and TA + MB).

Temperature rise was significantly attenuated compared to PL after both TA and TA + MB administrations ($P < 0.001$) and temperature rise was also suppressed by TA and TA + MB compared to MB ($P < 0.05$). However, there were no significant differences between TA and TA + MB.

3.2.4. Leucocyte migration

In exudate leucocyte numbers in PL-treated goats increased to a maximum of 24.5×10^9 cells L^{-1} at 24 h. By 48 h cell numbers (3.6×10^9 cells L^{-1}) were similar to 1 h values. The increase in cell numbers was significant between 3 and 36 h compared to 0 h values ($P < 0.05$). Exudate leucocyte numbers in MB, TA and TA + MB treated goats were similar to values obtained with PL (data not shown). Transudate leucocyte counts were very low and remained relatively constant at all sampling times up to 48 h in PL-treated goats and TA, TA + MB and MB treatments failed to modify cell number at any sampling time (data not shown). Blood

Table 3
Change in skin temperature (°C) and AUC for change in temperature with time (°C h) over exudate tissue cages after PL, TA, TA + MB and MB treatments (mean and SD, $n = 8$)

Time (h)	PL		TA		TA + MB		MB	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Skin temperature change								
1	0.68	0.62	0.35	0.34	0.45	0.57	0.78	0.40
3	0.92	0.79	0.65	0.68	0.45	0.11	0.56	0.34
6	1.05	1.56	0.65	0.71	0.65	0.51	0.95	0.48
9	1.81	1.84	1.08	0.74	1.08	0.42	1.55	0.65
12	1.95	1.36	0.68	0.54	0.70	0.57	1.45	0.57
24	2.05	1.56	0.75	0.42	0.85	0.23	1.55	0.51
30	1.56	0.99	0.65	0.42	0.75	0.28	1.05	0.17
36	0.95	0.68	0.55	0.51	0.55	0.34	0.80	0.34
48	0.85	0.65	0.40	0.34	0.32	0.40	0.35	0.62
AUC	65.2	47.7	25.5	26.1**	27.2	20.3*	48.4	22.9

* $P < 0.05$, difference from MB.
** $P < 0.001$, difference from PL.

leucocyte count was also relatively constant and there were no significant differences between the four treatments (data not shown).

3.3. Pharmacodynamic–pharmacokinetic modeling

Individual and mean values for pharmacodynamic (E_{max}, IC_{50} and N) and pharmacokinetic (K_{e0} and $t_{1/2}K_{e0}$) parameters derived by PK–PD modeling are presented in Table 4. For PGE_2, data were not available for PK–PD analysis in one goat and in another animal there was 100% inhibition of PGE_2 at all sampling times, so that the findings in this animal also could not be modeled. For inhibition of both serum TxB_2 and exudate PGE_2, efficacy (E_{max}) was high and IC_{50} was small, indicating high potency, particularly for PGE_2 inhibition. Excluding one outlier value, the IC_{50} potency ratio serum TxB_2:exudate PGE_2 was 12:1 but inter-animal variation was high (CV = 81%). Slopes (N) were 3.41 and 2.39 for inhibition of TxB_2 and PGE_2, respectively. Equilibration times for TxB_2 and PGE_2 inhibition were, respectively, short and relatively long; 0.70 h for TxB_2 and 16.5 h for PGE_2. However, the latter value was subject to marked inter-animal variation.

4. Discussion

4.1. Pharmacokinetics

The pharmacokinetics of TA after intramuscular dosing was described in 13 of 16 instances by a one-compartment model preceded by absorption. Similar findings have been reported in calves (Lees et al., 1998; Sidhu et al., 2005). Intravenously administered TA has been reported to fit a bi-compartmental model in calves, dogs and man (Landoni et al., 1996a; Lefebvre et al., 1997; McKellar et al., 1991; Pentikainen et al., 1981, 1984) and a three compartment model in horses (Jaussaud et al., 1991), but there are no comparable data for intravenous dosing in goats.

Absorption of TA from the intramuscular injection site was very rapid, as indicated by the short absorption half-life and time to attain maximum concentration. The terminal half-life of TA ($t_{1/2}K_{10}$ = 2.62 h) was shorter than previously reported in calves (8.22 h) (Lees et al., 1998; Sidhu et al., 2005) (Table 5). The relatively high volume of distribution (scaled to bioavailability) of TA is similar to previous findings in the calf (Landoni et al., 1996a; Lees et al., 1998; Sidhu et al., 2005). For example, Landoni et al. (1996b) reported a Vdarea of 0.98 L kg^{-1} after intravenous administration of tolfenamic acid in calves. In the present study the high value of 1.39 L kg^{-1} for Vdarea could reflect wholly or in part low bioavailability (Toutain and Bousquet-Melou, 2004). Because of high plasma protein binding of most NSAIDs, including TA, low values of distribution volume are usually obtained. For example, Vdarea values of 0.14 L kg^{-1} for TA in man and 0.35 and 0.16 L kg^{-1} for a related NSAID, flunixin, in dogs and horses, respectively, have been reported (Pentikainen et al., 1981; Hardie et al., 1985; Lees et al., 1987). In contrast, a high value for Vdarea has been reported for flunixin administered intravenously to calves (Landoni et al., 1995a). The cause of these relatively high volumes of distribution of TA in calves and goats and of flunixin in calves is unknown. A possible mechanism is ion trapping in saliva. The secretion of large volumes of alkaline

Table 4
Pharmacodynamic and pharmacokinetic parameters derived by PK–PD modeling for inhibition of serum TxB_2 and exudate PGE_2 in goats after administration of TA (mean and SD, n = 8 unless stated)

	Goat									
	1	2	3	4	5	6	7	8	Mean	SD
Serum TxB_2										
E_{max} (%)	64.3	97.2	55.0	31.3	64.2	65.1	99.5	78.6	69.4	22.3
IC_{50} (μg ml^{-1})	0.851	0.999	0.435	0.998	0.285	0.491	0.678	0.996	0.717	0.286
N	1.10	1.17	9.31	9.62	2.24	1.93	0.92	1.01	3.41	3.77
K_{e0} (h^{-1})	0.990	0.990	0.986	0.997	0.979	0.990	0.995	0.969	0.987	0.009
$t_{1/2}K_{e0}$ (h)	0.700	0.700	0.702	0.695	0.707	0.700	0.696	0.715	0.704	0.009
	Goat									
	1	2	3	4	6	8	Mean	SD		
Exudate PGE_2										
E_{max} (%)	61.1	78.2	99.9	99.5	99.8	99.9	89.7	16.5		
IC_{50} (μg ml^{-1})	0.030	0.077	0.052	0.002	0.116	0.162	0.073	0.059		
N	2.47	9.10	0.99	0.19	0.60	1.01	2.39	3.37		
K_{e0} (h^{-1})	0.015	0.040	0.995	0.023	0.993	0.170	0.373	0.485		
$t_{1/2}K_{e0}$ (h)	46.2	17.3	0.70	30.1	0.70	4.070	16.5	18.5		
IC_{50} TxB_2:IC_{50} PGE_2	28.4	13.0	8.4	499.0	4.2	6.1	12.0	9.5[a]		

[a] n = 5 (excluding goat 4).

Table 5
Comparison of pharmacokinetic properties of TA in calves and goats (mean and SD $n = 8$)

Parameter	Treatment							
	TA				TA + MB			
	Calf		Goat		Calf		Goat	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
C_{max} (μg ml^{-1})	2.14	0.27	1.64	0.85	1.64	0.23	1.13	0.28
T_{max} (h)	0.99	0.22	0.19	0.06	0.55	0.15	0.26	0.11
AUC (μg h ml^{-1})	27.38	3.23	6.45	3.34	16.80	4.88	3.97	1.22
CIB/F (L kg^{-1} h^{-1})	0.07	0.01	0.39	0.20	0.13	0.04	0.55	0.20
Vdarea/F (L kg^{-1})	0.87	0.12	1.39	0.54	1.17	0.18	1.73	0.40
$t_{1/2}K_{10}$ (h)	8.22	1.04	2.62	0.62	6.68	1.74	2.29	0.68

saliva is characteristic of ruminants, and weakly acidic drugs such as TA should achieve high concentrations in this fluid. Subsequent secondary absorption from the g.i.t. would be anticipated. However, McKellar et al. (1991) reported a large volume of distribution for TA in dogs, a non-ruminant species, and proposed enterohepatic cycling as a possible explanation. Subsequently, Priymenko et al. (1993) showed that 90% of administered TA in the dog was excreted in bile, conjugated with glucuronic and sulphuric acids. The enterohepatic re-cycling of TA has also been reported in man and the camel (Pentikainen et al., 1984; Wasfi et al., 1998).

It is widely recognised that co-administration of two drugs may affect the absorption, distribution, biotransformation and/or excretion of both agents (Benet and Sheiner, 1985). Indeed, co-administration in dogs of enrofloxacin and theophylline led to a decreased hepatic clearance and increased elimination half-life of theophylline (Intorre et al., 1995). With this exception it is surprising that pharmacokinetic interactions between NSAIDs and antimicrobial drugs have been little studied in veterinary medicine, given their frequent clinical use in combination. In this study there were no statistical differences in pharmacokinetic parameters and variables between TA and TA + MB treatments. However, there were trends towards reductions in C_{max} and AUC and an increase in CIB/F. In a similar study in calves the changes in C_{max}, AUC and CIB/F were similar but also achieved statistical significance (Sidhu et al., 2005). The lack of statistical significance in goats could be due to the power of the design or to the necessarily small number of animals used. The cause of the differences in TA pharmacokinetics produced by MB co-administration in calves was not determined.

The penetration into and elimination from tissue cage fluids (exudate and transudate) of TA were slow but nevertheless more rapid (penetration) and less persistent (elimination) than previously reported in calves (Sidhu et al., 2005). The relatively slow penetration but long persistence of TA in exudate is of therapeutic interest and supports the observation of Landoni et al. (1995a,b) and Lees et al. (1986, 1987) of a longer duration of action of NSAIDs than would be predicted from plasma concentrations. However, with the cylindrical cages used in this study and in the investigation of Sidhu et al. (2005) penetration was slower and persistence was longer than previously reported for TA in studies using spherical tissue cages (Lees et al., 1998; McKellar et al., 1994a), indicating that both rates of entry and exit are strongly model dependent. Persistence in this study is indicated by MRT which was approximately three times longer for both exudate and transudate than for serum. Thus, extrapolation of findings to biophases, based on tissue cage data, must be made with caution.

4.2. Pharmacodynamics

Carrageenan stimulation of granulation tissue within the cages provided a mild, localised, acute and reversible inflammatory reaction, which allowed elucidation of the magnitude and time course of the actions of TA at both molecular (inflammatory mediator) and clinical surrogate response (skin temperature) levels. Thus, both TA and TA + MB produced significant inhibition of synthesis of serum TxB_2 up to 12 h and exudate PGE_2 up to 48 h and suppression of skin temperature rise induced by carrageenan, but no inhibition of cell migration into exudate. Similar models have been used in cattle, horses, sheep and dogs to assess the effects of several NSAIDs including TA (Higgins and Lees, 1984a,b; Higgins et al., 1984; Lees et al., 1987, 1991; Landoni and Lees, 1995a,b; McKellar et al., 1994a,b; Cheng et al., 1997, 1998). The present data confirm and extend these findings to the goat.

In this study, between 9 and 48 h, mean values of exudate PGE_2 were slightly but *not* significantly higher after MB alone than after PL dosing. This observation is mentioned here only because a similar finding has been reported in calves, but in the latter species the increases were statistically significant (Sidhu et al., 2005).

NSAID/fluoroquinolone pharmacodynamic interactions have been reported previously. The combination of fenbufen with enoxacin produced convulsions in human patients (Wolfson and Hooper, 1989) and a single dose of fenbufen administered 10 min before several

fluoroquinolones produced tonic convulsions in mice and rats. No similar adverse CNS interactions have been reported in companion and farm animals (Neer, 1988). Indeed, no reference to a pharmacodynamic interaction between TA and MB in domestic or farm animal species could be found, except for one report in Beagle dogs (Bousquet-Melou et al., 1997). These authors reported that the co-administration of TA and MB induced neither clinical signs of seizures nor epileptiform electrocorticography signs, even at MB doses 10 times those recommended. In the present study the lack of effect of MB co-administration on the actions of TA at both molecular and surrogate clinical response levels is of therapeutic significance for those clinical cases when combination treatment is used.

4.3. PK–PD modeling

It has been established since 1991 that COX, the enzyme that generates eicosanoids such as PGE_2 and TxA_2, exists in two isoforms (COX-1 and COX-2), which exert a range of roles in physiological and pathological processes (Fu et al., 1990; Kujubu et al., 1991; Xie et al., 1991; Meade et al., 1993). More recently, a third isoform, COX-3, actually a splice variant of COX-1, has been identified in dog brain (Chandrasekharan et al., 2002). In the current investigation, serum TxB_2 (a stable breakdown product of TxA_2) was monitored ex vivo as an index of the time course of inhibition of COX-1 and in vivo inhibition of exudate PGE_2 is probably mainly and possibly solely due to COX-2 inhibition. Compared to calves, the inhibition of serum TxB_2 in goats was less pronounced and of shorter duration, whereas the level of PGE_2 inhibition was similar in the two species between 9 and 36 h but somewhat greater in goats at both earlier and later sampling times (Table 6).

Requirements for ex vivo and in vivo PK–PD modeling include graded responses and a sufficient number of sampling times. Both criteria were met in this investigation. PK–PD modeling of serum TxB_2 and exudate PGE_2 data provided values for the pharmacodynamic parameters: E_{max}, a measure of efficacy; IC_{50}, a measure of potency and slope N, a measure of sensitivity. It is of interest to compare the findings with those generated in calves in a similar study (Sidhu et al., 2005). In goats there was greater selectivity for COX-2 inhibition, as indicated by the ratio of 12:1. Therefore, in this model in this species TA may be classified as a preferential inhibitor of COX-2. In comparison, two studies in calves have yielded ratios of 1.8:1 and 1.4:1, suggesting a likely species difference (Landoni et al., 1996a, Sidhu et al., 2005). It is also of interest to compare differences between the two species, goat and calf, for inhibition of serum TxB_2 and exudate PGE_2. Based on mean values, the IC_{50} ratios, goat:calf, were 2.80:1 for the former and 0.23:1 for the latter. These potency differences are potentially important in that there may constitute a basis for species differences in clinical effectiveness, safety profile and dosage requirements.

Table 6
Comparison of mean percentage inhibition of serum TxB_2 and exudate PGE_2 in calves and goats ($n = 8$)

Time	TA		TA + MB	
	Calf	Goat	Calf	Goat
Percentage inhibition of serum TxB_2				
1	85	66	85	62
3	84	50	82	48
6	76	45	77	38
9	74	34	67	34
12	56	23	54	16
24	41	4	39	7
30	20	0	20	0
36	10	0	7	0
48	0	0	2	0
Percentage inhibition of exudate PGE_2				
1	1	41	23	35
3	39	85	27	80
6	69	83	70	84
9	82	82	84	86
12	78	76	82	81
24	88	78	77	79
30	70	77	76	69
36	57	66	66	53
48	48	73	57	66

Other authors have used in vitro assays to compare COX-1:COX-2 inhibition ratios (IC_{50}) for tolfenamic acid. Using washed canine platelets (COX-1) and a canine macrophage-like cell line (COX-2), Ricketts et al. (1998) reported a 15-fold selectivity of TA for COX-2. Kay-Mugford et al. (2000) used a canine macrophage cell line for assay of both isoforms and also reported moderate preferential inhibition of COX-2. In a canine whole blood assay tolfenamic acid possessed more than five times greater selectivity for COX-2 than for COX-1 (Wilson et al., 2004).

Finally, it should be noted that other authors have preferred to use an indirect response model for PK–PD modeling ex vivo and in vivo data (Lepist and Juko, 2004), rather than the effect compartment model used in this study. The choice of model should be determined by the cause of the delay (hysteresis) between plasma concentration and response to NSAIDs. In our model the cause is not known. It could be due either to a distributional delay to the site of action (pharmacokinetics) or to an indirect response with a significant time delay between action (inhibition of COX) and response (suppression of TxB_2 and PGE_2 synthesis). Alternatively, it could be due to both factors. Therefore, we have selected an effect compartment PK–PD model for consistency with earlier reports from this laboratory (Landoni and Lees, 1995a,b; Landoni et al., 1995a, 1996a; Sidhu et al., 2005).

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