ANTIINFLAMMATORY DRUGS

Pharmacodynamics, chiral pharmacokinetics and PK–PD modelling of ketoprofen in the goat

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There have been few studies of the pharmacodynamics of nonsteroidal antiinflammatory drugs (NSAIDs) using PK–PD modelling, yet this approach offers the advantage of defining the whole concentration–effect relationship, as well as its time course and sensitivity. In this study, ketoprofen (KTP) was administered intravenously to goats as the racemate (3.0 mg/kg total dose) and as the single enantiomers, $S(+)$ KTP and $R(-)$ KTP (1.5 mg/kg of each). The pharmacokinetics and pharmacodynamics of KTP were investigated using a tissue cage model of acute inflammation. The pharmacokinetics of both KTP enantiomers was characterized by rapid clearance, short mean residence time (MRT) and low volume of distribution. The penetration of $R(-)$ KTP into inflamed (exudate) and noninflamed (transudate) tissue cage fluids was delayed but area under the curve values were only slightly less than those in plasma, whereas MRT was much longer. The $S(+)$ enantiomer of KTP penetrated less readily into exudate and transudate. Unidirectional inversion of $R(-)$ to $S(+)$ KTP occurred. Both rac-KTP and the separate enantiomers produced marked inhibition of serum thromboxane B_2 (TxB_2) synthesis (*ex vivo*) and moderate inhibition of exudate prostaglandin E_2 (PGE_2) synthesis (*in vivo*); pharmacody-namic variables for $S(+)$ KTP were E_{max} (%) = 94 and 100; IC_{50} (µg/mL) = 0.0033 and 0.0030; N = 0.45 and 0.58, respectively, where E_{max} is the maximal effect, IC_{50} the plasma drug concentration producing 50% of E_{max} and N the slope of log concentration/effect relationship. The IC_{50} ratio, serum TxB_2:exudate PGE_2 was 1.10. Neither rac-KTP nor the individual enantiomers suppressed skin temperature rise at, or leucocyte infiltration into, the site of acute inflammation. These data illustrate for KTP shallow concentration–response relationships, probable nonselectivity of KTP for cyclooxygenase (COX)-1 and COX-2 inhibition and lack of measurable effect on components of inflammation.

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INTRODUCTION

Ketoprofen (KTP) is a 2-arylpropionate nonsteroidal antiinflam-matory drug (NSAID) containing a single asymmetric carbon atom. It, therefore, exists as two enantiomers, $R(-)$ and $S(+)$ KTP. For clinical use it is formulated as tablets and as an injection, both products containing the racemic (50:50) mixture of the two enantiomers. The eudismic ratio for cyclooxygenase (COX) inhibition, *S:R,* determined *in vitro,* clearly indicates that the $S(+)$ enantiomer is the eutomer (Caldwell *et al.*, 1988; Evans, 1992; Suesa *et al.*, 1993; Landoni *et al.*, 1996, 1997).

Enantiomeric differences in drug action are not surprising, as the body is a chiral environment, cell structures and enzymes being composed of D-sugars and L-amino acids. Moreover, for 2-arylpropionates in general, and for KTP in particular, stereospecific pharmacokinetics as well as stereoselective phar-macodynamics have been reported (Hutt & Caldwell, 1983; Evans, 1992). Pharmacokinetic differences may be due to enantiomer differences in volume of distribution, in rate of elimination, to metabolic inversion of the chiral centre or to more than one of these factors (Caldwell *et al.*, 1988; Foster & Jamali, 1988). For 2-arylpropionates, inversion is generally unidirectional involving $R(-)$ enantiomer conversion to the $S(+)$

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antipode. Enantioselective pharmacokinetics of KTP has not only been demonstrated in several species, including human, rat, rabbit, dog, cat, cow, sheep and horse, but also found to differ quantitatively between species (Abas & Meffin, 1987; Caldwell *et al.*, 1988; Foster & Jamali, 1988; Delatour *et al.*, 1993, 1994; Jaussaud *et al.*, 1993; Mauleon *et al.*, 1994; Landoni & Lees, 1995a,b, 1996b; Landoni *et al.*, 1995, 1999; Skeith *et al.*, 1996, Arifah *et al.*, 2001). There is overall predominance (measured as plasma concentration) after rac-KTP administration of $S(+)$ KTP in the rat, rabbit, dog, cat, monkey and horse (Abas & Meffin, 1987; Foster & Jamali, 1988; Delatour *et al.*, 1993, 1994; Jaussaud *et al.*, 1993; Mauleon *et al.*, 1994; Landoni & Lees, 1995a,b; Brink *et al.*, 1998) and of $R(-)$ KTP in sheep (Delatour *et al.*, 1994; Landoni *et al.*, 1999; Arifah *et al.*, 2001), whilst plasma concentrations of the two enantiomers are similar in humans and cattle (Sallustio *et al.*, 1988; Delatour *et al.*, 1994; Geisslinger *et al.*, 1995; Landoni *et al.*, 1995; Fuder *et al.*, 1997). This is in spite of the fact that chiral inversion of $R(-)$ to $S(+)$ KTP occurs in humans and cattle.

To our knowledge, there are no published pharmacokinetic data after administration of the separate KTP enantiomers in the goat and only one publication on pharmacokinetics after administration of the racemate (Musser *et al.*, 1998), which, however, reported only 'total drug' concentrations and pharmacokinetic variables derived from these concentrations. The use of total drug concentrations fails to recognize that racemates are mixtures of two drugs that almost invariably differ in pharmacokinetic and pharmacodynamic properties. The pharmacokinetic data generated in such studies have been described as 'highly sophisticated scientific nonsense' (Ariens, 1984; Campbell, 1990). There are no data on the pharmacodynamics of KTP in the goat. Because of its size and temperament, use of the goat permits serial blood and exudate sampling in the same animal and the use of cross-over designs. It is thus a useful species for investigating pharmacokinetic–pharmacodynamic (PK–PD) interrelationships in whole animal studies. The generation of pharmacokinetic and pharmacodynamic data may also be used to assist dosage schedule design for clinical use in the goat.

The objectives of the present investigation were to establish for rac-, $S(+)$ and $R(-)$ KTP in the goat: (i) enantioselective pharmacokinetics including penetration into inflamed (exudate) and noninflamed (transudate) tissue cage fluids; (ii) the magnitude and time course of inhibition *ex vivo* of serum thromboxane B_2 (TxB_2) in blood allowed to clot under standardized conditions; (iii) *in vivo* inhibition of prostaglandin E_2 (PGE_2) synthesis in inflammatory exudate; and (iv) effects on skin temperature and exudate leucocyte count at a site of acute inflammation. It is generally accepted that the TxB_2 generated when blood clots is entirely due to COX-1 activation. It is likely that most and possibly all of the PGE_2 generated at acute sites of inflammation is attributable to COX-2, although a contribution from COX-1 cannot be excluded. Even if there is a COX-1 contribution to the generation of PGE_2 in exudate, the present data have value in defining the extent and time course of inhibition of both isoenzymes at a site of inflammation. These *ex vivo* and *in vivo*

approaches are complementary to *in vitro* investigations of COX-1:COX-2 ratios of inhibition by NSAIDs, particularly in view of the wide variations reported in *in vitro* studies.

MATERIALS AND METHODS

The studies reported in this paper were approved by the RVC Ethics and Welfare Committee.

Animals

Twelve healthy female British Sannen goats, aged 3 years and weighing 53–99 kg, were used. They had been treated for gastrointestinal parasites and vaccinated against common infectious diseases before entry into the study.

Collection of tissue cage fluids

For the collection of inflammatory exudate and transudate, four spherical tissue cages were inserted subcutaneously in each goat under general anaesthesia, two on either side of the neck and each approximately equidistant from the jugular vein and spinal cord (Higgins *et al.*, 1984). The cage dimensions were: external diameter = 29 mm; internal diameter = 27 mm; number of holes = 10, each of 4.8 mm diameter; internal volume = 10.5 mL. After implantation, 5 weeks were allowed to permit wound healing and the growth of granulation tissue into each cage. To induce a mild, localized inflammatory response, 0.5 mL of 1% sterile carrageenan solution (Marine Colloids, Springfield, NJ, USA) was injected into a single cage at zero time. Exudate was harvested from a carrageenan-injected cage and noninflamed tissue cage fluid (transudate) was collected from a noninjected cage. In each period of each cross-over, exudate was collected from a previously noninjected cage. Tissue cage fluids have been described as comprising a deep peripheral compartment; they are not identical to but are in continuity with interstitial fluid (Clarke, 1989; Clarke *et al.*, 1989).

Experiment 1

A two-period cross-over study, such that each goat received intravenous (i.v.) rac-KTP (Ketofen 10%, Rhone Merieux, Harlow, Essex, UK) as a single dose at a dosage of 3 mg/kg (combined enantiomers) and placebo treatment, was undertaken in six goats. Two-week intervals were allowed between each period.

In period 1, 3 mg/kg rac-KTP was injected into the right jugular vein of three goats (over 10 sec) at time zero and three goats received an equivalent volume of normal saline (placebo) i.v. In period 2, treatments were reversed. Also at time zero, in each period of the cross-over study, each goat was given an intracaveal injection of carrageenan solution into one tissue cage. This cage was used to harvest exudate. A noninjected cage was used to collect transudate. In period 2, i.v. injections were administered into the left jugular vein and the two tissue cages not used in period 1 were used to harvest exudate and transudate.

Samples of blood for determination of plasma KTP enantiomer concentration (5 mL in Li-heparin monovettes, Sarstedt, Leicester, Leicestershire, UK) were collected from a jugular vein before (−30 min), at 5, 10, 15, 20, 30 and 40 min, and at 1, 1.5, 2, 3, 4, 6, 9, 12, 24, 30, 36 and 48 h after drug or placebo administration. Blood samples were placed on ice, centrifuged at $2200 \times g$ and 4 °C for 10 min and the supernatant plasma pipetted into polystyrene tubes. Samples were stored at −20 °C. Blood samples (2.5 mL) were collected in ethylenediaminetetraacetic acid (EDTA) monovettes for the measurement of leucocyte counts at exudate sampling times (see below). Further blood samples (2.5 mL) for *ex vivo* measurement of serum TxB_2 production (in blood allowed to clot in glass tubes at 37 °C for 60 min) were withdrawn before (−30 min) and at 1, 2, 3, 4, 6, 9, 12, 24, 30, 36 and 48 h after placebo and KTP administration. Samples were then placed on ice and serum separated by centrifugation at $2200 \times g$ and 4 °C for 10 min. Samples were stored at −20 °C prior to TxB_2 analysis.

Exudate and transudate samples (1.2 mL) were collected at times of 1, 3, 6, 9, 12, 24, 30, 36 and 48 h for measurement of KTP enantiomer and PGE_2 concentrations. Each sample was collected into tubes containing 10 μg BW540C (Wellcome Foundation, Beckenham, Kent, UK), a dual COX, 5-lipoxygenase inhibitor to prevent artifactual generation of eicosanoids. A 0.1-mL aliquot was used for determination of total leucocyte count. The remainder was centrifuged to remove cells, divided into aliquots and stored at −20 °C prior to analysis. Skin temperature over the exudate tissue cages was monitored at exudate sampling times using an Horiba IT-330 Infrared thermometer (Horiba Instruments Ltd., Breackmills, Northampton, UK).

Experiment 2

A three-way cross-over study (using a Latin square design), such that each goat received i.v. $S(+)$ and $R(-)$ KTP (1.5 mg/kg) and placebo treatment, was undertaken in six adult healthy female goats. The rac-KTP dose (3 mg/kg) used in Experiment 1 is the recommended dose for cattle; therefore, half of the combined dose was the appropriate dosage for each enantiomer in Experiment 2. Two-week intervals were allowed between each period. In period 1, two goats received 1.5 mg/kg $S(+)$ KTP and two received 1.5 mg/kg $R(-)$ KTP i.v. into the right jugular vein (over 10 sec at time 0). Two goats received an equivalent volume of normal saline (placebo) i.v. Each enantiomer was prepared as a 5% solution in a 50:50 mixture of ethanol and water for injections from pure $S(+)$ and $R(-)$ enantiomers supplied by Laboratorios Menarini (Barcelona, Spain). The levels of enantiomeric purity were 99.8 and 99.5% for $S(+)$ and $R(-)$ enantiomers, respectively. In periods 2 and 3, treatments were changed so that each animal received each treatment in sequence.

At time zero, in each period, to generate exudate, each goat received an injection into a single tissue cage of sterile carrageenan solution. A noninjected cage was used to collect transudate. In periods 2 and 3, cages not used previously to harvest exudate were used to generate and collect exudate.

Exudate and transudate samples (1.2 mL) were collected 1, 3, 6, 9, 12, 24, 30 and 36 h after drug or placebo dosing as described for Experiment 1.

Samples of blood for determination of plasma KTP enantiomer concentrations (5 mL in Li-heparin monovettes) were collected from a jugular vein before, at 5, 10, 15, 20, 30 and 40 min, and at 1, 1.5, 2, 3, 4, 6, 9, 12, 24, 30 and 36 h after drug or placebo administration. Further samples (2.5 mL) were collected in EDTA monovettes for the measurement of blood leucocyte counts at exudate sampling times. Blood samples (2.5 mL) for *ex vivo* measurement of serum TxB_2 production were withdrawn before (−30 min) and at 1, 2, 3, 4, 6, 9, 12, 24, 30 and 36 h after dosing. The processing and storage of samples were as described for Experiment 1. Skin temperature measurements over exudate cages were taken as for Experiment 1.

Analytical methods and measurements

Concentrations of KTP enantiomers in plasma were measured by high-pressure liquid chromatography (HPLC) using the method of Foster & Jamali (1987) as modified by Landoni & Lees (1995b). The HPLC system used comprised a system controller (Waters 600E, Milford, MA, USA), a tunable absorbance detector (Waters 484, Milford, MA, USA), a Shimadzu autoinjector (SIL-6A, Kyoto, Japan), a system controller (SCL-6A, Kyoto, Japan) and an integrator (Waters 746, Milford, MA, USA). The precolumn and column used were Lichrospher RPC_{18} 5 μm and Lichrosorb RPC_{18} 7 μm (250×4 mm), respectively. Percentage recoveries for $S(+)$ and $R(-)$ KTP were 90 ± 5 and $92 \pm 4\%$, respectively. Coefficients of variation over the concentration range 0.01–10 μg/mL of each enantiomer were <12%. The limit of quantification was 0.05 μg/mL.

Serum TxB_2 and exudate PGE_2 concentrations were measured by radioimmunoassays (Higgins & Lees, 1984). The limits of detection and quantification were 0.05 and 0.08 ng/mL for both eicosanoids. The coefficient of variation at a concentration of 0.05 was 11.5 ± 1.5 and for 10 ng/mL it was $8.6 \pm 2.6\%$.

Skin temperature over the carrageenan-injected cage was monitored using an Horiba IT-330 Infrared thermometer.

Leucocyte counts in whole blood, exudate and transudate were measured using a Coulter Counter (Coulter Electronics Ltd., Luton, Bedfordshire, UK).

Pharmacokinetic and pharmacodynamic analyses

Plasma concentration–time data for individual goats were analysed using PC NONLIN compartmental programs by non-linear least squares regression analysis (SCI Software, Statistical Consultants Inc, Lexington, KY, USA). Minimum Akaike Information Criteria Estimates were applied to discriminate the best fitting model and improved fit of data was achieved by re-weighting. In every instance, for all drug administrations to all animals the data fitted the bicompartmental model equation, $C_p = Ae^{-\alpha t} + Be^{-\beta t}$. Pharmacokinetic terms are defined later.

The extent of chiral inversion of $R(-)$ KTP was determined using the formula:

Inversion extent = [*AUC S*(+) KTP after *R*(−) KTP dosing

$\times$ dose of *S*(+) KTP]/[*AUC S*(+) KTP after

S(+) KTP dosing $\times$ dose of *R*(−) KTP]

PK–PD modelling for inhibition of serum TxB_2 and exudate PGE_2 was carried out by extended nonlinear least-squares regression analysis, using the MK model program (Biosoft, Cambridge, Cambridgeshire, UK). In view of the hysteresis present in the relationship between NSAID concentration and measured effects, a mamillary compartment model was applied (Landoni & Lees, 1995a,b). In this model, the active compartment is assumed to receive a negligible amount of drug and to have a negligible volume (Dahlstrom *et al.*, 1978; Sheiner *et al.*, 1979; Holford & Sheiner, 1981, 1982). Initial estimates of E_{max} and IC_{50} were calculated graphically from simulated concentrations. In order to relate plasma concentration of *S*(+) KTP to observed effects a Hill plot was applied:

$$E = E_0 \pm E_{max} \cdot C_e^N)/IC_{50}^N + C_e^N)$$

where E_0 is the control value, E_{max} the maximal effect, C_e the apparent drug concentration in the effect compartment, EC_{50} the plasma drug concentration producing 50% of E_{max} and N the slope of the log concentration/effect relationship.

From previous studies, it is assumed that inhibition 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 primarily to an action on COX-2, although some contribution from COX-1 inhibition cannot be excluded.

Statistical analysis

The significance of differences between drug- and placebo-treated goats was assessed using analysis of variance and, where significant differences were established, Fisher's multi-comparisons test was used. Differences were accepted as significant for $P < 0.05$.

RESULTS

Experiment 1

Plasma concentrations of *R*(−) and *S*(+) KTP were similar at all sampling times after i.v. administration of rac-KTP (Fig. 1a). Respective *AUC* ratios were 6.81 and 5.15 µg h/mL. For both *S*(+) and *R*(−) KTP the plasma concentration–time relationship for each of the six animals fitted a bi-compartmental model. For *S*(+) KTP, both distribution ($t_{1/2\alpha}$) and elimination ($t_{1/2\beta}$) half-lives were relatively short, 0.18 and 1.79 h, respectively, and *MRT* was 1.28 h (Table 1). Corresponding values for *R*(−) KTP were 0.19 h ($t_{1/2\alpha}$), 1.87 h ($t_{1/2\beta}$) and 1.36 h (*MRT*). Volume of the central compartment (V_c) and volume of distribution at steady state (V_{dss}) were relatively low, 0.13 and 0.39 L/kg, respectively, for *S*(+) KTP and 0.10 and 0.29 L/kg for *R*(−) KTP. Clearance (CIB) was rapid, 0.30 L/(kg h) for *S*(+) KTP and 0.23 L/(kg h) for *R*(−) KTP. The K_{12}:K_{21} ratios indicated a faster
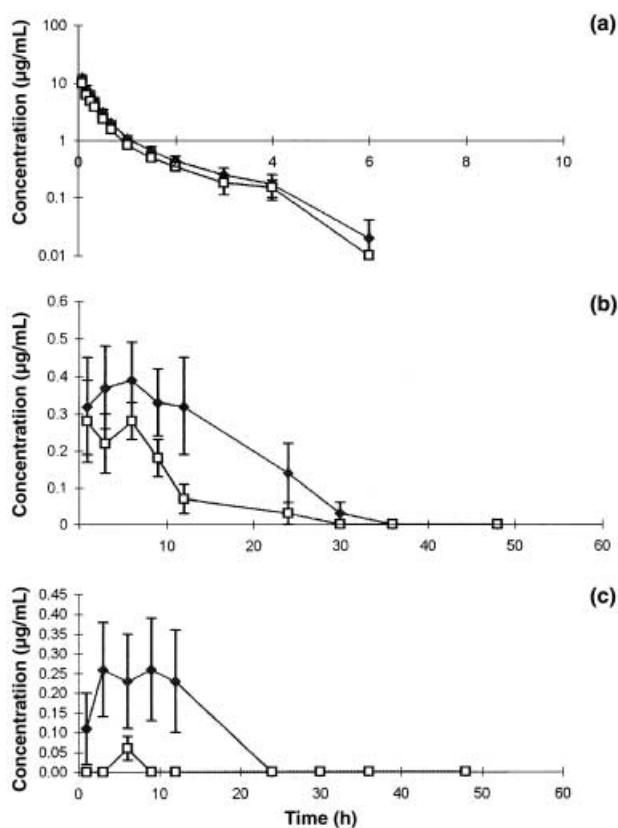

Fig. 1. Concentration–time relationships for *R*(−) KTP (closed symbols) and *S*(+) KTP (open symbols) after i.v. administration of rac-KTP (3.0 mg/kg). Values are mean ± SEM ($n = 6$): (a) semi-logarithmic plot of plasma concentration; (b) arithmetic plot of exudate concentration; (c) arithmetic plot of transudate concentration.

rate of drug transportation of both enantiomers from the central to the peripheral compartment than from the peripheral to central compartment (Table 1). Differences in pharmacokinetic parameters between *S*(+) and *R*(−) KTP were not statistically significant.

In exudates concentrations of *S*(+) KTP were low compared to those in plasma. Exudate *AUC* for this enantiomer was approximately half plasma *AUC*, whilst exudate *R*(−) KTP *AUC* was only slightly less than the corresponding plasma *AUC* (Tables 1 & 2). *S*(+) KTP in exudate was represented by a plateau between 1 and 6 h, decreasing thereafter, whereas *R*(−) KTP concentration in exudate was relatively constant between 1 and 12 h (Fig. 1b). The differences in exudate *AUC* and C_{max} values for the two enantiomers indicates predominance of *R*(−) KTP.

In transudate, quantifiable concentrations of *S*(+) KTP were present in two animals only and at a single time only, 6 h (Fig. 1c). *R*(−) KTP concentrations were higher, with measurable concentrations in three of six animals at 12 h (Fig. 1c). Area under curve was 5.22 µg h/mL, which was slightly less than exudate *AUC* for *R*(−) KTP.

Serum TxB_2 concentration after placebo administration was relatively constant, mean values falling within the range 140–210 ng/mL (Fig. 2). Serum TxB_2 synthesis was virtually abolished up to 6 h and significantly inhibited up to 12 h after

Table 1. Mean (± SEM, $n = 6$) pharmacokinetic parameters of $S(+)$ KTP and $R(-)$ KTP after i.v. administration of rac-KTP (3 mg/kg) to goats

	$S(+)$ KTP		$R(-)$ KTP	
Parameter	Mean	SEM	Mean	SEM
A (µg/mL)	11.59	1.49	14.64	1.96
B (µg/mL)	0.88	0.13	1.00	0.12
α (h^{-1})	3.90	0.23	3.75	0.30
β (h^{-1})	0.45	0.11	0.42	0.06
$t_{1/2\alpha}$ (h)	0.18	0.01	0.19	0.01
$t_{1/2\beta}$ (h)	1.79	0.24	1.87	0.28
V_c (L/kg)	0.13	0.01	0.10	0.01
V_{dss} (L/kg)	0.39	0.07	0.29	0.05
K_{10} (h^{-1})	2.45	0.28	2.35	0.26
K_{12} (h^{-1})	1.21	0.19	1.16	0.26
K_{21} (h^{-1})	0.70	0.10	0.66	0.07
CIB (L/kg/h)	0.30	0.03	0.23	0.02
AUC (µg h/mL)	5.15	0.46	6.81	0.66
MRT (h)	1.28	0.20	1.36	0.29

A, B, Y-axis intercept terms; α, distribution rate constant; β, elimination rate constant; $t_{1/2\alpha}$, distribution half-life; $t_{1/2\beta}$ elimination half-life; V_c volume of central compartment; V_{dss} volume of distribution at steady state; K_{10} central compartment elimination rate constant; K_{12} rate constant for passage from central to peripheral compartment; K_{21} rate constant for passage from peripheral to central compartment; C1B, body clearance; AUC, area under concentration versus time curve; MRT, mean residence time.

Table 2. Mean (±SEM, $n = 6$) exudate and transudate pharmacokinetic parameters of $S(+)$ KTP and $R(-)$ KTP after i.v. administration of rac-KTP (3 mg/kg) to goats

	Exudate				Transudate	
	$S(+)$ KTP		$R(-)$ KTP		$R(-)$ KTP	
Parameter	Mean	SEM	Mean	SEM	Mean	SEM
C_{max} (µg/mL)	0.37	0.07	0.58	0.08	0.58	0.06
T_{max} (h)	3.83	1.01	6.33	2.01	9.00	2.12
AUC (µg h/mL)	2.57	0.63	6.00	1.89	5.22	0.95
MRT (h)	6.38	1.33	7.84	1.88	6.65	0.22

C_{max} maximum concentration; T_{max} time of maximum concentration; other terms are described in Table 1.

administration of rac-KTP ($P < 0.05$), in spite of the fact that $S(+)$ KTP and $R(-)$ KTP concentrations in plasma were <LOQ at 9 h and thereafter. At 24 h and subsequent times, no inhibition was obtained.

Mean peak PGE$_2$ concentration occurred at 12 h after placebo administration (Fig. 3). Exudate PGE$_2$ was reduced by rac-KTP at all times between 6 and 48 h, but the reduction was significant only at 9, 12 and 24 h ($P < 0.05$).

The mean increases in skin temperature over tissue cages, following i.v. rac-KTP, were not statistically different from temperature rises after placebo administration (data not shown).
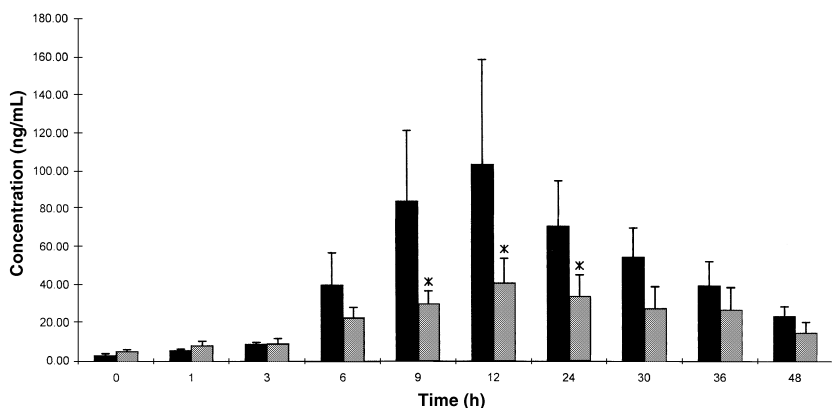
Leucocyte numbers in whole blood, exudate and transudate were not statistically significant after i.v. rac-KTP dosing compared to placebo treatment (data not shown).
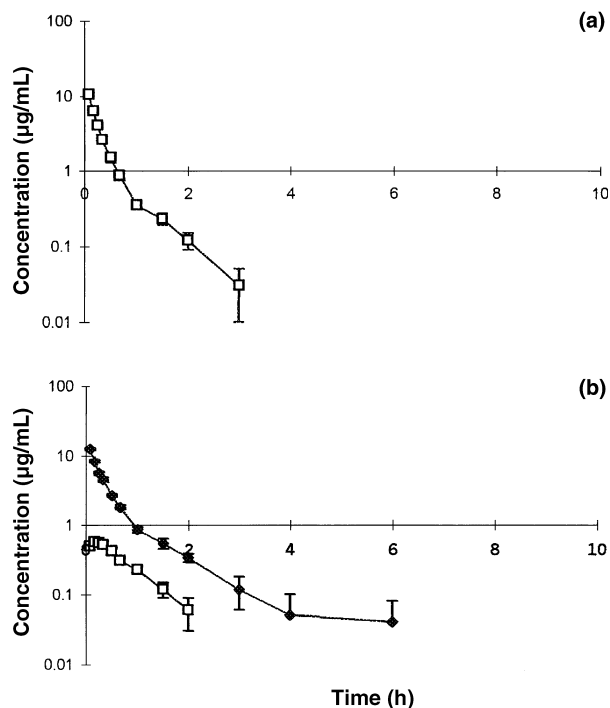
Experiment 2

Similar plasma concentration–time profiles were obtained after separate i.v. administration of $R(-)$ and $S(+)$ KTP, each at a dosage of 1.5 mg/kg, to those obtained for the racemate at a total dose of 3 mg/kg (Figs 1a & 4). The data for both enantiomers for all six goats fitted a bi-compartmental model. The MRT was relatively short for both enantiomers, but particularly for $S(+)$ KTP for which MRT was lower than that obtained after administration of rac-KTP. This is probably explained by chiral inversion of $R(-)$ to $S(+)$ KTP after administration of the racemate. In the single enantiomer study, the rate of inversion was shown to be rapid, the plasma concentration of $S(+)$ KTP after administration of $R(-)$ KTP falling within the range 0.43–0.56 µg/mL at the five sampling times between 5 and 30 min and declining thereafter. The percentage extent of inversion was 15.2 ± 1.7. Because of the chiral inversion of $R(-)$ to $S(+)$ KTP in the racemate study (Experiment 1), but not in this experiment, derived pharmacokinetic variables must be interpreted with caution. For example, the clearance of $S(+)$ KTP is 0.43 L/kg/h after $S(+)$ enantiomer dosing and the apparent clearance is 0.30 L/kg/h after rac-KTP dosing. A correction factor, to allow for $R(-)$ KTP inversion, has not been obtained, as the $S(+)$ KTP thus formed is not part of the administered dose.

As previously demonstrated for rac-KTP, for the single enantiomers V_c and V_{dss} were low and CIB was rapid,
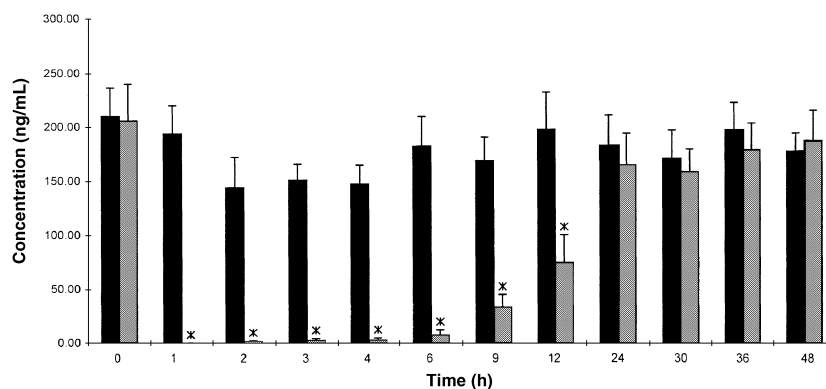
Fig. 2. Time course of serum TxB$_2$ synthesis in placebo (black columns) and rac-KTP (stippled columns) treated goats. Values are mean ± SEM ($n = 6$). Statistical difference from placebo ($P < 0.05$) is indicated by asterisks.

Table 3. Mean (±SEM, $n = 6$) pharmacokinetic parameters of $S(+)$ KTP and $R(-)$ KTP in goats after separate i.v. administration of each enantiomer (1.5 mg/kg)

Parameter	$S(+)$ KTP		$R(-)$ KTP	
	Mean	SEM	Mean	SEM
A (μg/mL)	16.14	1.09	15.86	0.65
B (μg/mL)	1.28	0.27	3.50	1.11
α (h^{-1})	6.59	0.64	6.27	0.87
β (h^{-1})	1.21	0.24	1.13	0.28
$t_{1/2\alpha}$ (h)	0.11	0.01	0.12	0.01
$t_{1/2\beta}$ (h)	0.66	0.09	0.92	0.33
V_c (L/kg)	0.09	0.01	0.08	0.01
V_{dss} (L/kg)	0.17	0.02	0.19	0.05
K_{10} (h^{-1})	4.89	0.41	3.47	0.35
K_{12} (h^{-1})	1.30	0.28	1.84	0.26
K_{21} (h^{-1})	1.62	0.30	2.10	0.64
CIB (L/kg/h)	0.43	0.03	0.27	0.01
AUC (μg h/mL)	3.62	0.23	5.71	0.30
MRT (h)	0.40	0.05	0.77	0.27

Pharmacokinetic terms are defined in Table 1.

Fig. 4. Semi-logarithmic plots of plasma concentration–time relationships for $R(-)$ KTP (closed symbols) and $S(+)$ KTP (open symbols), after i.v. administration of the separate enantiomers (1.5 mg/kg). Values are mean ± SEM ($n = 6$): (a) administration of $S(+)$ KTP; (b) administration of $R(-)$ KTP.

particularly for $S(+)$ KTP (Table 3). Similarly, as expected as a consequence of chiral inversion, AUC for the S-enantiomer was somewhat lower than the corresponding value obtained after administration of rac-KTP.

After administration of $S(+)$ KTP, there were no measurable drug concentrations in exudate in two animals at any time. In the remaining goats, $S(+)$ KTP concentrations were low between 1 and 9 h and undetectable by 12 h (Fig. 5a). The mean AUC was 0.76 μg h/mL and C_{max} was 0.18 μg/mL (Table 4). Both values were lower than corresponding values after rac-KTP administration, 2.57 μg h/mL and 0.37 μg/mL, respectively (Table 2). After $R(-)$ KTP dosing, the mean exudate concentra-

tion was maximal at 1 h and undetectable in most animals by 24 h (Fig. 5b).

After dosing with the individual enantiomers, AUC values, $S(+)$ KTP (0.76 μg h/mL) and $R(-)$ KTP (4.23 μg h/mL) indicate predominance of the latter in exudate. $S(+)$ KTP was not detected in exudate after $R(-)$ KTP administration.

Similar findings were obtained for transudate concentrations after separate administration of $S(+)$ KTP to those obtained for the racemate (Figs 1c & 6b). Mean AUC and C_{max} values indicated predominance of $R(-)$ KTP over $S(+)$ KTP in transudate. Also, for both enantiomers transudate concentrations were lower than those in exudate (Table 4).

Serum TxB$_2$ concentration after placebo administration was relatively constant (Fig. 7). Synthesis of this eicosanoid was significantly inhibited up to 12 h after administration of both enantiomers and additionally at 24 h after administration of $R(-)$ KTP ($P < 0.05$).

In animals receiving placebo, the peak PGE$_2$ concentration in exudate occurred at 12 h (Fig. 8). Prostaglandin E$_2$ synthesis
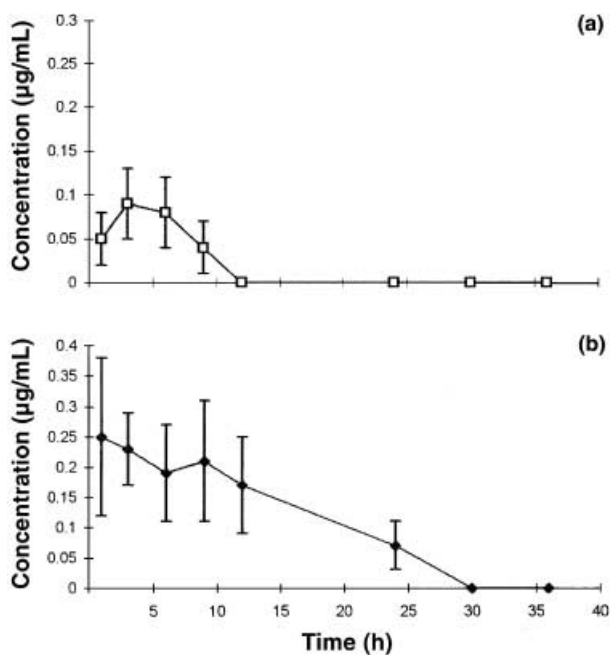
Fig. 5. Arithmetic plots of exudate concentration–time relationships for $R(-)$ KTP (closed symbols) and $S(+)$ KTP (open symbols), after i.v. administration of the separate enantiomers (1.5 mg/kg). Values are mean ± SEM ($n = 6$): (a) administration of $S(+)$ KTP; (b) administration of $R(-)$ KTP.
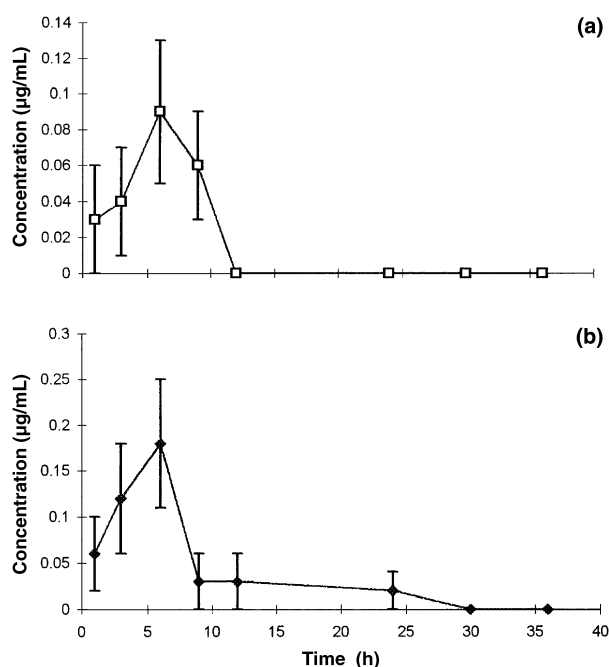
Table 4. Mean (±SEM, $n = 6$) exudate and transudate pharmacokinetic parameters of $S(+)$ KTP and $R(-)$ KTP in goats after separate i.v. administration of each enantiomer (1.5 mg/kg)

	Exudate				Transudate			
	$S(+)$ KTP		$R(-)$ KTP		$S(+)$ KTP		$R(-)$ KTP	
Parameter	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
C_{max} (µg/mL)	0.18	0.02	0.45	0.10	0.14	0.03	0.28	0.04
T_{max} (h)	4.00	1.00	3.40	1.34	4.50	0.71	4.40	0.94
AUC (µg h/mL)	0.76	0.27	4.24	1.72	0.70	0.24	1.29	0.73
MRT (h)	4.58	0.50	6.89	1.35	5.75	0.40	5.72	1.24

Pharmacokinetic terms are defined in Tables 1 and 2.

was significantly inhibited at 6, 9, 12 and 24 h by both $S(+)$ and $R(-)$ KTP ($P < 0.05$).

The sigmoidal E_{max} equation provided pharmacodynamic constants for $S(+)$ KTP. Values are presented in Tables 5 & 6 for serum TxB_2 inhibition and exudate PGE_2 inhibition, respectively. To facilitate inter-species comparisons, the tables indicate corresponding values for the horse, calf and sheep obtained in previous studies, using similar models and techniques. For the goat and these three species the slopes of the concentration–response relationships were variable but in all cases relatively shallow. Equilibration half-times were shorter for TxB_2 than PGE_2 inhibition, reflecting the delay in penetration of $S(+)$ KTP into the deep peripheral compartment (exudate). For both eicosanoids in all four species the low IC_{50} values reflected the

Fig. 6. Arithmetic plots of transudate concentration–time relationships for $R(-)$ KTP (closed symbols) and $S(+)$ KTP (open symbols), after i.v. administration of the separate enantiomers (1.5 mg/kg). Values are mean ± SEM ($n = 6$): (a) administration of $S(+)$ KTP; (b) administration of $R(-)$ KTP.

high potency of $S(+)$ KTP COX-1:COX-2 inhibition ratios ranged from 0.48 (horse) to 3.38 (sheep), indicating some variation between species.

The increases in skin temperature over the tissue cages after i.v. $S(+)$ KTP and $R(-)$ KTP administration were not significantly different from those after placebo administration (data not shown). Leucocyte numbers in whole blood, exudate and transudate were not statistically significant after i.v. $S(+)$ and $R(-)$ KTP dosing compared to placebo treatment (data not shown).

DISCUSSION

Enantioselective pharmacokinetics of KTP has been described in several animal species, including humans, cats, dogs, rats, rabbits, calves, horses and sheep (Abas & Meffin, 1987; Jamali, 1988; Sallustio *et al.*, 1988; Delatour *et al.*, 1993, 1994; Landoni & Lees, 1995a,b, 1996a,b; Landoni *et al.*, 1995, 1999; Castro *et al.*, 2000; Arifah *et al.*, 2001), but there are no published data on the pharmacokinetics and pharmacodynamics of KTP enantiomers in the goat. The present study demonstrated the enantioselective pharmacokinetics of KTP in this species, $R(-)$ KTP being the predominant enantiomer in exudate and transudate, whilst similar concentrations of the two enantiomers were present in plasma. Moreover, $R(-)$ KTP was chirally inverted to $S(+)$ KTP, a phenomenon previously demonstrated for other species. Reported percentage extents of inversion are 5.9 (male sheep, Corriedale
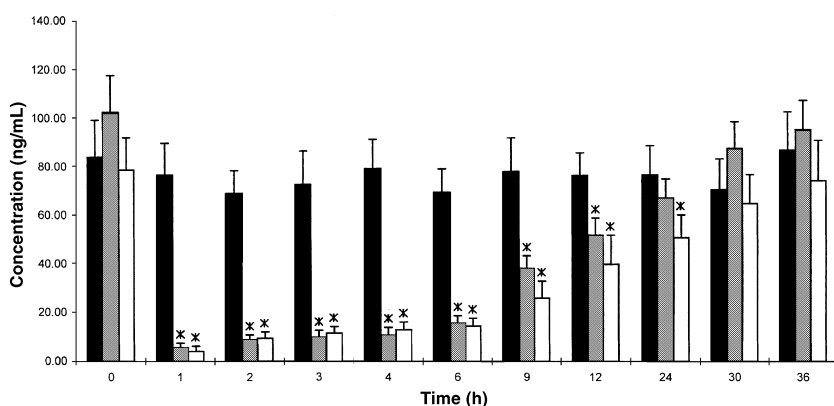
Fig. 7. Time course of serum TxB_2 synthesis in placebo (black columns), $S(+)$ KTP (stippled columns) and $R(-)$ KTP (white columns) treated goats. Values are mean ± SEM ($n = 6$). Statistical difference from placebo ($P < 0.05$) is indicated by asterisks.
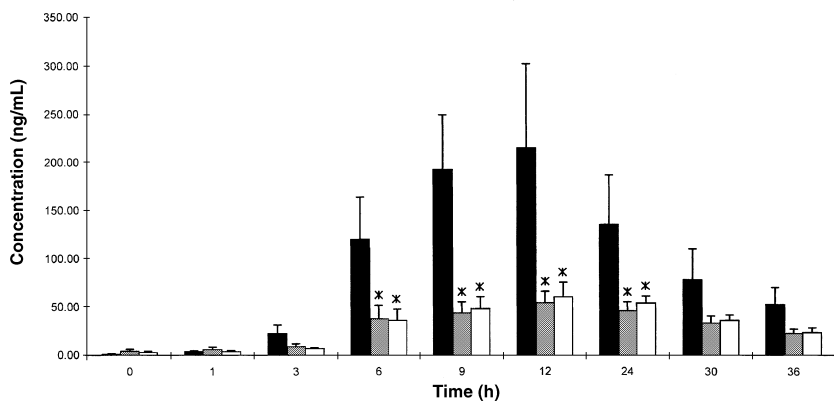

Fig. 8. Time course of exudate PGE_2 synthesis in placebo (black columns), $S(+)$ KTP (stippled columns) and $R(-)$ KTP (white columns) treated goats. Values are mean ± SEM ($n = 6$). Statistical difference from placebo ($P < 0.05$) is indicated by asterisks.

Table 5. Mean ($n = 6$) pharmacodynamic parameters for inhibition of serum TxB_2 by rac-KTP and $S(+)$ KTP after i.v. administration

	Horse		Calf		Sheep		Goat	
Parameter (units)	Rac-KTP*	$S(+)$ KTP[†]	Rac-KTP[‡]	$S(+)$ KTP[§]	Rac-KTP[¶]	$S(+)$ KTP[¶]	Rac-KTP**	$S(+)$ KTP**
E_{max} (%)	114	102	99	103	127	127	110	94
IC_{50} (µg/mL)	0.061	0.016	0.118	0.047	0.034	0.025	0.129	0.0033
N	0.42	1.30	2.04	3.46	1.12	0.47	0.62	0.45
K_{eo} (h^{-1})	0.34	0.21	0.42	0.26	0.51	0.46	0.32	0.22
$T_{1/2}K_{eo}$ (h)	3.02	3.15	2.05	2.78	1.85	2.14	2.22	3.15

E_{max} maximal effect; IC_{50}, plasma drug concentration producing 50% of E_{max}; N, Hill coefficient; K_{eo} rate constant of equilibration of drug in effect compartment; $T_{1/2}K_{eo}$ half-life of equilibration of drug in effect compartment.
* Landoni & Lees (1995b); [†] Landoni & Lees (1996b); [‡] Landoni *et al.* (1995); [§] Landoni & Lees (1995a); [¶] Landoni *et al.* (1999); ** This study.

cross), 13.8 (female sheep, Dorset cross), 15.0 (cat), 31.0 (calf) and 48.8 (horse) (Landoni & Lees, 1995a, 1996b; Landoni *et al.*, 1999; Arifah *et al.*, 2001; Lees *et al.*, 2002).

Mean $R(-):S(+)$ *AUC* ratios after rac-KTP administration were 1.3 and 2.3 for plasma and exudate, respectively, and for transudate *AUC* was too low to record for $S(+)$ KTP, giving a ratio >5.0. The greater penetration of $R(-)$ KTP than $S(+)$ KTP into both exudate and transudate, relative to plasma concentrations, also occurred after administration of the separate enantiomers. As enantiomers have similar physico-chemical properties, these differences will not be due, for example, to differences in lipid solubility and passive diffusion rates. A possible explanation is a greater degree of binding to plasma

protein of the $S(+)$ enantiomer. However, this explanation is not favoured by the slightly greater volume of distribution of $S(+)$ KTP after rac-KTP dosing and the similar distribution volumes of $S(+)$ and $R(-)$ KTP after single enantiomer administration. Elucidation of the mechanism of enantioselective distribution into tissue cage fluids, therefore, requires further study.

Also of interest is the tendency of both enantiomers to penetrate more readily into exudate than transudate. This has been demonstrated for KTP and other NSAIDs in several species and may be due to a high level of plasma protein binding. This would be expected to facilitate exudate penetration, as protein (with bound drug) leaks into exudate from plasma (Landoni & Lees, 1995a,b, 1996b; Landoni *et al.*, 1995, 1999). An

Table 6. Mean ($n = 6$) pharmacodynamic parameters for inhibition of exudate PGE_2 by rac-KTP and $S(+)$ KTP after i.v. administration and COX-1 : COX-2 inhibition ratios

Parameter (units)	Horse		Calf		Sheep		Goat	
	Rac-KTP	$S(+)$ KTP	Rac-KTP	$S(+)$ KTP	Rac-KTP	$S(+)$ KTP	Rac-KTP	$S(+)$ KTP
E_{max} (%)	100	94	91	99	92	94	112	100
IC_{50} (µg/mL)	0.057	0.033	0.086	0.042	0.012	0.0074	0.039	0.0030
N	3.68	1.78	1.85	2.79	2.97	1.60	1.40	0.58
K_{eo} (h^{-1})	0.27	0.16	0.19	0.12	0.16	0.32	0.09	0.11
$T_{1/2}K_{eo}$ (h)	3.44	4.32	4.43	6.10	5.04	4.22	6.93	6.30
COX 1 : COX 2, IC_{50} ratio	1.07	0.48	1.37	1.12	2.83	3.38	3.30	1.10

Pharmacodynamic terms and references are described in Table 5.

additional potential factor is the vasodilation of arterioles that occurs in acute inflammation.

After i.v. administration of rac-KTP, mean $t_{1/2\beta}$ values were relatively short, 1.79 and 1.87 h for $S(+)$ and $R(-)$ KTP, respectively. Even shorter $t_{1/2\beta}$ values of 0.42 h for both enantiomers have been previously reported in the calf by Landoni *et al.* (1995) and of 0.63 h for both enantiomers in sheep (Arifah *et al.*, 2001). V_{dss} of both enantiomers in goats at steady-state was low. Previous work has shown that KTP is highly bound (>99%) to plasma proteins (Debruyne *et al.*, 1987; Jamali & Brocks, 1990), and this probably explains the limited distribution.

In vitro investigations in previous studies have demonstrated not only that $S(+)$ KTP is the eutomer but that the potency ratio for COX inhibition has varied from 33:1 to >5000 in a range of *in vitro* assays in human cells and equine synoviocytes (Hayball *et al.*, 1992, 1993; Suesa *et al.*, 1993; Landoni *et al.*, 1996). It is, therefore, of interest that in the goat both rac-KTP and the separate enantiomers produced profound and long-lasting inhibition of serum TxB_2. Indeed, $R(-)$ KTP was at least as effective as $S(+)$ KTP in inhibiting serum TxB_2. This may be due in part to $S(+)$ KTP formed from the $R(-)$ enantiomer by chiral inversion. However, it is also likely to imply that the potency ratio $S(+):R(-)$ KTP is less in the goat than that reported in *in vitro* assays, using cells from other species. *In vitro* whole blood assays in the goat are required to confirm the postulated low enantiomer potency ratios in this species.

Given the limited penetration of both KTP enantiomers but particularly $S(+)$ KTP into exudate, the degree and persistence of inhibition of PGE_2 illustrates the high potency of KTP as a COX inhibitor. Inhibition was still significant at 24 h after rac-KTP dosing, by which time concentrations of both enantiomers had decreased to low levels. Moreover, as for inhibition of serum TxB_2, exudate PGE_2 was inhibited to a similar degree after $R(-)$ as after $S(+)$ KTP administration, in spite of the fact that $S(+)$ KTP concentrations in exudate were extremely low after dosing with both enantiomers. Potency overall is, therefore, high but potency ratios, $R(-):S(+)$ KTP, for COX inhibition cannot be determined *ex vivo* or *in vivo,* because of the inversion of $R(-)$ to $S(+)$ KTP. To establish potency ratios for the goat, it will be necessary to perform studies *in vitro*.

The landmark discovery of Vane (1971) that COX inhibition by NSAIDs was the principal mechanism whereby they exert their therapeutic (analgesic, antiinflammatory and antipyretic) and toxic effects, including gastrointestinal and in some circumstances renal effects, has been supported by much subsequent data, even though it is recognized that NSAIDs affect many other biochemical pathways. The COX hypothesis of NSAID action was further advanced by the discovery in 1990–1991 of two COX isoforms, now termed COX-1 and COX-2 (Fu *et al.*, 1990; Kujubu *et al.*, 1991; Xie *et al.*, 1991). Cyclooxygenase-1 is a constitutive enzyme, present in most cells and concerned with a range of 'housekeeping' functions, including gastro- and renoprotection and blood clotting, whilst COX-2, although present as a constitutive enzyme in some cells, is induced at sites of inflammation to generate pro-inflammatory prostaglandins. It is now widely held that inhibition of COX-1 accounts for most of the side-effects of NSAIDs, whilst inhibition of COX-2 accounts partly or wholly for therapeutic effects. Relative potency for inhibition of COX isoforms is usually determined in *in vitro* assays and expressed as the IC_{50} ratio for COX-1:COX-2 inhibition. There are problems with this approach, when used in isolation. First, for all NSAIDs investigated, reported values vary widely, depending on experimental conditions. Most researchers now accept that whole blood assays provide the most physiologically and pathologically relevant data. Even so, Warner *et al.* (1999) report IC_{50} ratios (COX-1:COX-2) for KTP in human cell assays of 0.016 in a whole blood assay and 0.19 in a whole blood modified assay (our calculation from their data). Although these values differ by more than 10-fold, both suggest that KTP has some selectivity for COX-1 inhibition. A KTP IC_{50} COX-1:COX-2 ratio for human isoenzymes in infected *Spodoptera frugiperda* cells of 0.0125 also suggests selectivity of KTP for COX-1 (Cromlish & Kennedy, 1996). Secondly, as clinical efficacy of NSAIDs is likely to require virtually complete inhibition of pro-inflammatory prostaglandins, it is more relevant to determine IC_{80} or even IC_{95} values than IC_{50} ratios in considering dosages for clinical use (*vide infra*).

As well as the wide variation with experimental conditions in reported COX-1:COX-2 inhibition ratios for NSAIDs, there may be species differences. In recent canine studies, Brideau *et al.* (2001) and Simmons *et al.* (personal communication) reported COX-1:COX-2 ratios of 0.6 and 0.57, respectively, for rac-KTP in

canine whole blood assays. Using separated canine platelets (COX-1) and a macrophage-like cell line (COX-2), Ricketts *et al.* (1998) reported a COX-1:COX-2 inhibition ratio of 0.232 for rac-KTP. On the basis of these three studies and the findings of Warner *et al.* (1999), KTP can be classified as a nonselective COX inhibitor in the dog and as COX-1 selective (or at least preferential) in man.

Most literature reports describing COX-1:COX-2 ratios for 2-arylpropionates such as KTP are based on the racemate. This is unfortunate, as the relative potency of the enantiomers of chiral compounds for COX-1 and COX-2 inhibition is a major consideration. We have conducted studies using single enantiomers of KTP in canine whole blood assays. IC_{50} for COX-1:COX-2 was 0.64 for $S(+)$ KTP and 0.44 for $R(-)$ KTP (Lees *et al.*, 2000). It will be of interest, particularly in view of the levels of inhibition of serum TxB_2 and exudate PGE_2 obtained after $R(-)$KTP dosing in this study, to establish the activities of each enantiomer against both COX-1 and COX-2 in the goat in future *in vitro* studies.

The $S(+)$ KTP IC_{50} ratio for serum TxB_2:exudate PGE_2 of 1.10 obtained in this study indicates that it is probably a nonselective COX inhibitor in the goat, as suggested previously in the horse, calf and sheep, for which corresponding ratios were 0.48, 1.12 and 3.38, respectively (Table 6). These *ex vivo* (serum TxB_2) and *in vivo* (exudate PGE_2) approaches to studies of COX inhibition complement and may be preferable to *in vitro* studies, in that they are more likely to simulate physiological and pathological conditions. Moreover, these findings suggest that there may be small species differences in ratios of serum TxB_2:PGE_2 inhibition by $S(+)$ KTP, although the magnitude of the differences may be too small to reflect clinically significant species differences in toxicity for equal therapeutic effects. Possible differences between species in IC_{50} values for both COX-1 and COX-2 inhibition require further study to determine whether they are attributable to differences in protein binding or interspecies potency differences or both.

Previous studies in our laboratory have demonstrated hysteresis associated with the action of KTP (and other NSAIDs) (Landoni & Lees, 1995a,b, 1996b; Landoni *et al.*, 1999). This has been shown in goats, horses, calves and sheep by measuring equilibration half-time ($T_{1/2}K_{eo}$) in studies similar to the present investigation. The present models and techniques thus generate relevant *ex vivo* and *in vivo* data on time course of eicosanoid inhibition, which is not generally available from *in vitro* studies. For $S(+)$ KTP, $T_{1/2}K_{eo}$ values for serum TxB_2 inhibition in these species were 2.22, 3.15, 2.78 and 2.14 h, respectively, and corresponding values for exudate PGE_2 inhibition were 6.30, 4.32, 6.10 and 4.22 h (this study and Landoni & Lees, 1995a,b, 1996b; Landoni *et al.*, 1995, 1999).

The marked and persistent inhibition of serum TxB_2 and moderate inhibition of exudate PGE_2 in the goat contrast with the failure of both rac-KTP and the individual enantiomers to suppress two components of the inflammatory response, leucocyte infiltration into exudate and skin temperature rise over tissue cages. The latter provides an indirect measure of the heat (a cardinal sign) generated in acute inflammation.

Regarding leucocyte infiltration into exudate, it is a common finding in our laboratory that, whilst NSAIDs inhibit chemotaxis *in vitro,* they do not usually reduce leucocyte infiltration *in vivo.* This is not unexpected, as prostaglandins probably exert little effect on leucocyte movement at concentrations generated *in vivo* at sites of inflammation (Lees *et al.*, 1986; Dawson *et al.*, 1987).

A possible explanation for failure to inhibit skin temperature rise is that, at the dosage used, there was incomplete inhibition of exudate PGE_2. It should be noted that there is no equal proportionality between inhibition of COX and suppression of both the cardinal signs of inflammation and the underlying microcirculatory events. This was shown in the early studies of Higgs *et al.* (1976, 1981), who found that, in rats, NSAID ED_{50} values for both suppression of inflammatory swelling and infiltration of leucocytes into acute inflammatory sites were much higher than ED_{50} values for PGE_2 inhibition. In a previous study in this laboratory, IC_{50} values for inhibition of serum TxB_2 and PGE_2 in inflammatory exudate by flunixin in the horse was determined (Landoni & Lees, 1995b). On the other hand, Toutain *et al.* (1994), using the same drug at the same dosage, also in the horse, reported IC_{50} values for effect on stride length in a Freund's adjuvant model of arthritis. The inhibition ratios IC_{50} stride length:IC_{50} serum TxB_2 and IC_{50} stride length:IC_{50} exudate PGE_2 were 26:1 and 49:1, respectively. From the slopes of the concentration–response relationships it was calculated that to achieve 50% effect on stride length requires approximately 90% inhibition of synthesis of pro-inflammatory prostaglandins. These data are compatible with the present findings of moderate COX inhibition in inflammatory exudate and the absence of effect on skin temperature rise. They also support the proposal of Warner *et al.* (1999) that it might be more appropriate in determining clinically relevant NSAID concentrations to measure concentrations producing 80% inhibition of COX-1 and COX-2. The present findings suggest that use of *in vivo* PGE_2 inhibition in the carrageenan tissue cage model of inflammation to predict dosages required for clinical efficacy should be based on 80 or even 95% blockade. In this respect two points should be noted. First, in the goat slopes of *ex vivo* (serum TxB_2) and *in vivo* (exudate PGE_2) inhibition relationships for $S(+)$ KTP were very shallow, 0.45 and 0.58, respectively, so that much higher concentrations (and doses) will be required for 80–95% than for 50% eicosanoid inhibition. Secondly, slopes for TxB_2 and PGE_2 inhibition by $S(+)$ KTP were almost parallel in the horse, calf and goat, but not in sheep, so that IC_{50} and IC_{80} ratios serum TxB_2:exudate PGE_2 for the former three species will be similar though not identical. In sheep, on the other hand, the IC_{80} ratio will be greater than the IC_{50} ratio.

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