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PHARMACODYNAMICS AND CHIRAL PHARMACOKINETICS OF CARPROFEN IN CALVES

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SUMMARY

The non-steroidal anti-inflammatory drug, carprofen, was administered intravenously as the racemate at a dose rate of 0.7 mg kg^{-1} to six Friesian bull calves aged 8–10 weeks. Anti-inflammatory properties were indicated by attenuation of temperature rise at sites of intradermal injection of the irritants, carrageenin and dextran, but responses were not statistically significant at most recording times. Carrageenin- and dextran-induced swelling were not significantly reduced by carprofen. Carprofen reduced *ex vivo* serum thromboxane B_2 synthesis but this effect was also not significant at most sampling times. Enantioselective pharmacokinetics of carprofen was demonstrated, plasma concentrations of the R(−) enantiomer predominating at all sampling times. It is concluded that inhibition of cyclo-oxygenase is unlikely to be the sole mechanism of action of carprofen in calves.

KEYWORDS: Carprofen; enantiomers; calves; pharmacokinetics; chiral.

INTRODUCTION

Carprofen is a non-steroidal anti-inflammatory drug (NSAID) used in horses, dogs and cattle. It contains a single chiral centre and therefore exists in two isomeric forms, the S(+) and R(−) enantiomers. The product available for use in veterinary medicine is the racemic mixture. Laboratory animal studies have demonstrated that the two enantiomers have differing potencies for various biological activities (Gaut *et al.*, 1975). Thus, the eudismic ratio of carprofen (S:R) is >16 for

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prostaglandin (PG) synthesis inhibition in sheep and >23 for inhibition of platelet aggregation (Gaut *et al.*, 1975). Laboratory animal studies have also shown that the pharmacokinetics of the enantiomers of 2-arylpropionic acids such as carprofen differ significantly, as a result of differences in metabolism, excretion and distribution. Moreover, chiral inversion of some 2-arylpropionic acids from R(−) to S(+) enantiomers has been demonstrated in man and other species (Caldwell *et al.*, 1988; Evans, 1992; Delatour *et al.*, 1993, 1994). It is therefore important, in studies attempting to relate anti-inflammatory drug activity to pharmacokinetic disposition, to determine the time-course of concentrations of each enantiomer in biological fluids such as plasma and inflammatory exudate.

It is also necessary to undertake studies on the pharmacokinetics of carprofen enantiomers in each of the target species for which clinical use is intended, since there will inevitably be differences between species. Thus, studies in this laboratory have shown that, in contrast to most NSAIDs of the 2-arylpropionic acid class, plasma concentrations of the R(−) enantiomer exceed those of the S(+) enantiomer in the horse and the magnitude of the difference increases with time (Lees *et al.*, 1991). Moreover, it has been shown that these differences are not due to chiral inversion of the S(+) to the R(−) enantiomer (P. Delatour and P. Lees, unpublished results). An absence of chiral inversion of R(−) and S(+) carprofen enantiomers has also been established in the dog (McKellar *et al.*, 1994).

Previous studies of carprofen pharmacokinetics in the horse (Lees *et al.*, 1994), sheep (Welsh *et al.*, 1992) and cattle (Ludwig *et al.*, 1989; Lohuis *et al.*, 1991), following administration of the racemic mixture, have measured total drug concentration rather than concentrations of the enantiomers. Recently, Delatour *et al.* (1993) compared the chiral pharmacokinetics of ketoprofen and carprofen in man, dog, sheep and pig, but similar studies in cattle have not been reported. The objectives of the present investigation were:

(1) to establish plasma concentration-time relationships and determine pharmacokinetic parameters in calves for each of the two enantiomers of carprofen following intravenous (i.v.) injection of the racemic mixture at a dose rate of 0.7 mg kg^{-1};
(2) to establish the time-course of inhibition of serum thromboxane (TX)B_2 synthesis (a biochemical marker of inhibition of platelet cyclo-oxygenase) by circulating concentrations of carprofen;
(3) to determine the magnitude and time-course of inhibition by carprofen of the temperature rise and oedematous swelling produced by intradermal (i.d.) injection of the mild irritants carrageenin and dextran.

MATERIALS AND METHODS

Animals and experimental design

A cross-over experimental design in six healthy Friesian bull calves, aged 8–10 weeks and weighing 55–83 kg, was used. In part 1 of the study, three calves received carprofen (0.7 mg kg^{-1}) by rapid i.v. injection at time 0. Three further calves received no treatment (controls). Carprofen was supplied as a 5% solution

of the racemic mixture for parenteral use (Zenecarp Injection, Grampian Pharmaceuticals Ltd). In part 2 of the cross-over, those animals receiving drug in part 1 received no treatment and *vice versa*. A 14-day interval between the two parts of the cross-over was allowed.

At time 0, each of the six calves received in areas of the neck, clipped and shaved 24 h previously, 0.1 ml injections of sterile carrargeenin solutions (strengths 0.25% and 0.0625% w/v) and 0.1 ml injections of sterile dextran solutions, molecular weight (MW) 249 000 (strengths 4% and 1%, w/v). The lesion swelling (volume) and skin temperature at the centre of each injection site were monitored at pre-determined times up to 72 h. Since the lesions were approximately elliptical in shape, the volume was determined from the formula for half an ellipse, $V=\frac{2}{3}\pi r_1 r_2 r_3$ where r_1, r_2 and r_3 are the radii. The latter were determined by measuring two perpendicular diameters with vernier callipers, and the change in skinfold thickness measured using spring gauge callipers provided the third radius. Lesion skin temperature was measured using a direct reading infrared thermometer (Horiba IT-330 Infrared Thermometer, Miyanohigas).

Blood sampling

Blood samples for determination of plasma carprofen concentration (Li-heparin monovettes, Sarstedt Ltd.) were collected before and at pre-determined times up to 144 h following carprofen administration. Samples were placed immediately on ice and centrifuged within 30 min of collection for 15 min at 4°C and 2500 ***g***. The supernatant plasma was stored at −20°C until extraction and analysis by high pressure liquid chromatography (HPLC). Blood samples for measurement of *ex vivo* serum TXB_2 concentration (blood was allowed to clot at 37°C for 60 min) were taken before and at pre-determined times after dosing up to 72 h. Serum was harvested by centrifugation at 4°C and 2500 ***g*** for 10 min. Samples were stored at −20°C until analysis for TXB_2 using a previously described radio-immunoassay method (Higgins & Lees, 1984; Lees *et al.*, 1987).

Measurement of carprofen enantiomer concentrations in plasma

(a) Extraction. Plasma (0.5 ml) was spiked with the internal standard (S(+)-naproxen) at a final concentration of 5 μg ml^{-1}. The sample was acidified with 1 ml potassium phthalate buffer (pH 5.2) and extracted twice with 8 ml diethyloxide. The two organic portions were combined and evaporated to dryness on a heating plate at 60°C under a nitrogen stream.

(b) Derivatization. The crude extract was submitted to an adaptation of the derivatization procedure described by Foster and Jamali (1987), which binds both enantiomers of carprofen with a chiral reagent, L-leucinamide. The two diastereomers produced were then separated on a classical reverse phase HPLC column.

(c) Chromatographic conditions. The column used was a RP8, ODS Ultraspher column (Beckman), 15 cm length, 0.4 cm internal diameter. The mobile phase was an acetonitrile:water mixture; gradient from 50% to 20% in 2 min; flow rate 1 ml min^{-1}. Detection (UV-2050 Varian) was at 300 nm wavelength. Under these conditions, the retention times were:

Internal standard = 6.50 min
R(−)-carprofen = 9.70 min
S(+)-carprofen = 10.70 min

The calibration was linear (R=1.00) for concentrations between 0.1 and 10 μg ml^{-1} of each enantiomer. The absolute recoveries were 98.6% for R(−) and 98.3% for S(+)-carprofen.

(d) Separation and quantitation of carprofen enantiomers. A HPLC trace illustrating the separation of R(−) and S(+) enantiomers of carprofen on plasma samples collected from a calf between 5 min and 144 h after dosing with racemic carprofen (0.7 mg kg^{-1}, i.v.) is presented in Fig. 1. Total carprofen concentration was calculated by summation of the separate enantiomer concentrations.

Statistical evaluation

Data are expressed as mean±standard error (SE) of the mean values. The significance of differences between mean values for untreated and carprofen-treated calves was determined using an analysis of variance (ANOVA) programme. Differences were considered to be statistically significant for $P<0.05$.

Pharmacokinetics

Pharmacokinetic constants were calculated for individual animals:

A (μg ml^{-1}) = zero time intercept (distribution phase).
B (μg ml^{-1}) = zero time intercept (elimination phase).
α (h^{-1}) = slope (distribution phase).
β (h^{-1}) = slope (elimination phase).
$t_{\frac{1}{2}\alpha}$ (h) = half-life (distribution phase).
$t_{\frac{1}{2}\beta}$ (h) = half-life (elimination phase).
Vc (ml kg^{-1}) = volume of central compartment.
Vd_{area} (ml kg^{-1}) = volume of distribution (area method).
Vd_{ss} (ml kg^{-1}) = volume of distribution (steady state).
Cl (ml h^{-1} kg^{-1}) = clearance.
MRT (h) = mean residence time.
Cp48h (μg ml^{-1}) = plasma concentration at 48 h.
AUC (μg h^{-1} ml^{-1}) = area under curve (0–144 h) for plasma concentration *vs* time.

RESULTS

Pharmacokinetics of carprofen and its enantiomers

Mean (±SEM) concentrations of total carprofen and each enantiomer in plasma are presented in Table I. A semi-logarithmic plot of plasma carprofen concentration (total and separate enantiomers) is presented in Fig. 2. The mean ratio of concentrations, expressed as percentages, in the first plasma sample (5 min) was 52.8:47.2 (R:S) and the concentration ratio changed slowly with time. Thus, the

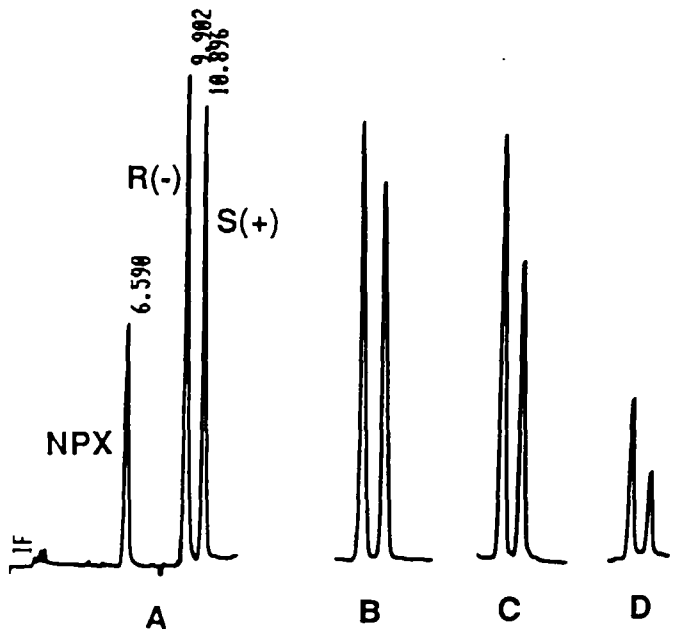

Fig. 1. Chiral HPLC trace illustrating the separation of R(−) and S(+)-carprofen enantiomers on plasma samples collected from a calf between 1 min and 144 h after i.v. administration of racemic carprofen (0.7 mg kg^{-1}). A, internal standard (naproxen) and calibration R:S=50:50; B, 5 min, R:S=53:47; C, 48 h, R:S=58:42; D, 144 h, R:S=72:28. Retention times were 6.590 min (naproxen), 9.902 min [R(−)-carprofen] and 10.896 min [S(+)-carprofen].

enantiomeric ratio, R:S, had widened slightly to 54.3:45.7 at 12 h and had increased further to 60.8:39.2 at 72 h. By 144 h the concentration ratio was 67.3:32.7.

Pharmacokinetic parameters are reported in Table II. In all six calves the data fitted a two-compartment open model. Distribution half-life values were similar for the two carprofen enantiomers but elimination half-life was lower for the S(+) than the (R−) enantiomer and mean differences were statistically significant. Vc was similar for the two enantiomers but Vd_{area} and Vd_{ss} were significantly greater for the S(+) enantiomer and, in addition, clearance was more rapid. On the other hand, MRT and Cp(48h) were significantly greater for the R(−) enantiomer.

Mean values for area under the plasma concentration-time curve (μg h ml^{-1}) from 0 to 144 h were 259.9 (total carprofen), 150.4 (R-enantiomer) and 109.6 (S-enantiomer). Hence, the R-enantiomer contributed 57.9% and the S-enantiomer 42.4% of the total AUC, differences which were statistically significant.

Lesion skin temperatures

Mean±SEM values of skin temperature change at sites of injection of carrageenin (0.0625% and 0.25%) are presented in Fig. 3. Following i.d. administration of the low concentration of carrageenin to non-drug-treated calves, mean skin temperature was increased at all recording times between 1 and 72 h. The maximum rise in mean temperature was 3.55°C at 8 h. In calves receiving carprofen, skin

Table I
Plasma carprofen concentrations (μg ml^{-1}) in calves following i.v. dosing of racemic carprofen at 0.7 mg kg^{-1} (mean±SEM, n=6)

Time (h)	*Total carprofen*	*R(−) carprofen*	*S(+) carprofen*
0.083	9.69±0.59	5.12±0.33	4.57±0.26
0.167	9.19±0.31	4.91±0.17	4.28±0.15
0.250	8.74±0.46	4.69±0.24	4.05±0.22
0.375	8.13±0.26	4.42±0.14	3.71±0.12
0.500	7.41±0.31	4.04±0.17	3.37±0.14
0.750	6.88±0.26	3.78±0.13	3.11±0.13
1	6.37±0.20	3.50±0.10	2.87±0.10
2	5.48±0.22	2.99±0.11	2.49±0.11
3	4.85±0.15	2.61±0.07	2.24±0.09
4	4.71±0.19	2.53±0.09	2.18±0.10
6	3.88±0.13	2.10±0.06	1.78±0.07
8	3.84±0.10	2.09±0.05	1.75±0.05
12	3.59±0.12	1.95±0.06	1.65±0.05
24	2.94±0.09	1.62±0.05	1.32±0.05
30	2.50±0.11	1.40±0.06	1.10±0.06
36	2.35±0.12	1.33±0.06	1.01±0.06
48	1.98±0.12	1.15±0.06	0.83±0.06
72	1.39±0.10	0.85±0.06	0.55±0.04
120	0.78±0.02	0.50±0.01	0.28±0.02
144	0.62±0.02	0.41±0.01	0.20±0.02

Weight=60.0±1.8 kg (part 1); 75.2±2.2 kg (part 2).
Age=8 weeks (part 1); 10 weeks (part 2).

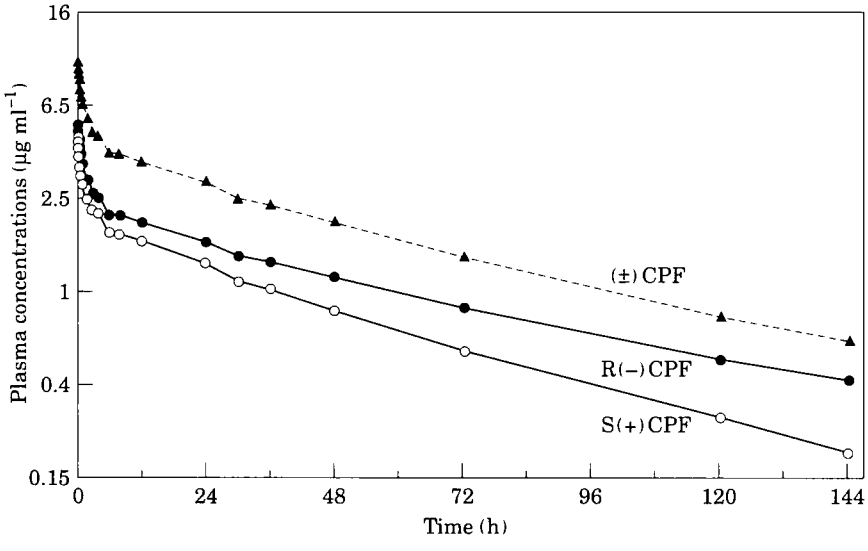

Fig. 2. Semi-logarithmic plot of plasma carprofen concentrations [total, R(−) and S(+)] *vs* time following i.v. administration of racemic carprofen (0.7 mg kg^{-1}) at time 0. Each point represents the mean value for six calves.

Table II
Pharmacokinetic variables following racemic carprofen administration (0.7 mg kg^{-1} i.v.) (mean±SEM, n=6)

Variable	*Total carprofen*	*R(−) carprofen*	*S(+) carprofen*
A (μg ml^{-1})	5.69±0.64	3.01±0.24	2.70±0.27
B (μg ml^{-1})	4.53±0.46	2.37±0.09	2.13±0.06**
α (h^{-1})	1.25±0.28	1.04±0.19	1.42±0.32
β (h^{-1})	0.016±0.001	0.014±0.001	0.019±0.001**
$t_{\frac{1}{2}\alpha}$ (h)	0.828±0.264	0.883±0.247	0.742±0.261
$t_{\frac{1}{2}\beta}$ (h)	43.4±2.3	49.7±3.9	37.4±2.4**
Vc (ml kg^{-1})	69.8±4.5	66.3±3.8	73.8±5.1
Vd_{area} (ml kg^{-1})	151.8±4.3	144.8±4.8	162.3±2.5**
Vd_{ss} (ml kg^{-1})	154.7±2.7	147.2±3.4	162.8±2.2***
MRT (h)	63.5±2.9	72.5±5.1	54.1±3.4***
Cl (ml h^{-1} kg^{-1})	2.492±0.171	2.073±0.145	3.075±0.221***
Cp (48h) (μg ml^{-1})	1.98±0.25	1.15±0.06	0.83±0.06***
AUC_{0-144} (μg h ml^{-1})	260±72	150±58	110±58**

*P<0.05, **P<0.01, ***P<0.001 comparison of R(−) and S(+) enantiomers.

temperatures were also increased but the magnitude of the change was less at 11 out of 12 measuring times. A secondary rise in skin temperature was recorded in both treated and non-treated calves at 30 h. Attenuation of the temperature rise by carprofen was statistically significant at three recording times (4, 8 and 12 h, P< 0.05) and differences from non-treated calves approached statistical significance at two other recording times (1 and 3 h; P=0.08 at both times).

Skin temperature also increased following i.d. injection of the high concentration of carrageenin (0.25%) and, overall, the rises were similar to those occurring with the lower concentration (Fig. 3). Carprofen tended to attenuate the carrageenin-induced rise in skin temperature; mean values were lower in drug-treated animals at nine out of 12 recording times and the maximum temperature rise was reduced from 3.77°C to 2.63°C. However, attenuation of the skin temperature rise by carprofen was statistically significant at only one recording time, 1 h (P <0.05), although differences also approached significance at 8 h (P=0.08).

Mean skin temperature was increased following i.d. administration of the low concentration of dextran (1%) in non-drug-treated calves at all measuring times up to 72 h. The maximum rise was 3.32°C at 8 h (Fig. 4). Carprofen administration reduced the rise in skin temperature at all 12 recording times and this was statistically significant at 1, 2, 4 and 36 h (P<0.05, P<0.01, P<0.03, P<0.05, respectively). The reductions approached significance at 30 h (P<0.08).

The higher concentration of dextran (4%) also increased skin temperature, the maximum mean value of 3.52°C occurring at 8 h (Fig. 4). A secondary rise was recorded at 30 h in both treatment groups. At most, but not all, recording times skin temperature rise was reduced by carprofen administration by about 40%. Attenuation of the skin temperature rise achieved statistical significance at only one recording time (4 h, P=0.02), but differences approached significance at 12 h (P=0.07).

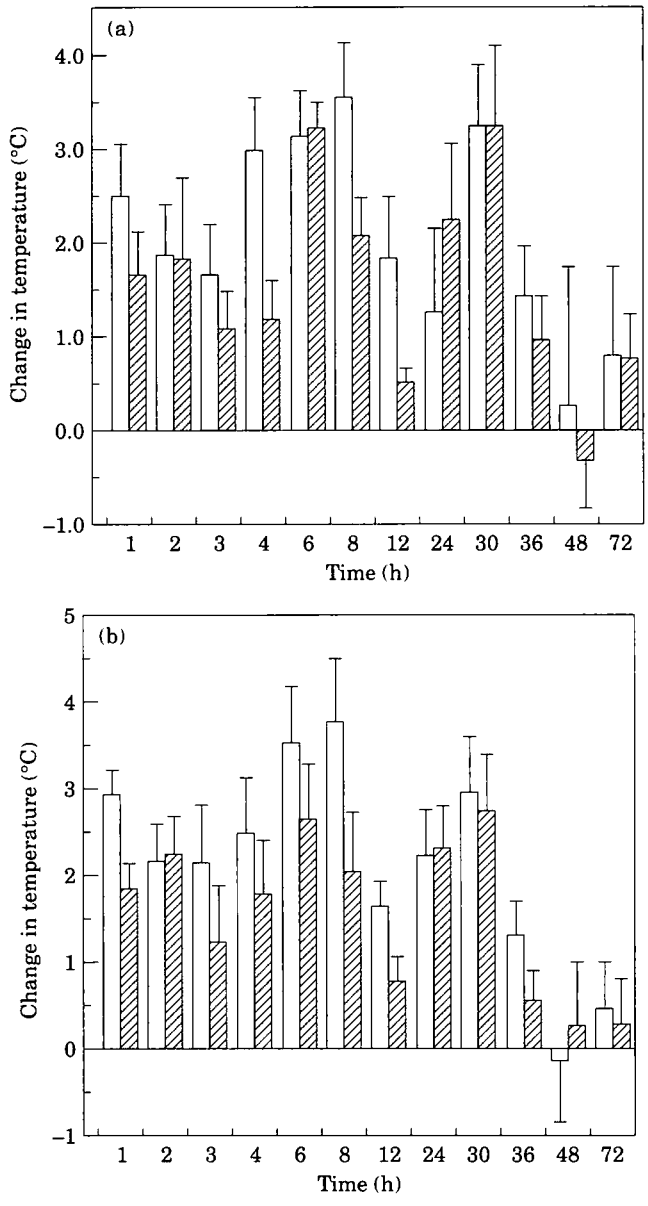

Fig. 3. Change in skin temperature from control values at sites of injection of (a) 0.0625% carrageenin and (b) 0.25% carrageenin in calves that received no drug treatment (□) or carprofen (0.7 mg kg^{-1} i.v.) (▨). Carprofen and carrageenin were both injected at time 0. Each column represents the mean±SEM for six calves.

Lesion volume

The low concentration of carrageenin (0.0625%) produced measurable lesions in control calves up to 12 h after administration, the peak volume occurring at 3 h. Lesion volumes in carprofen-treated calves did not differ significantly from

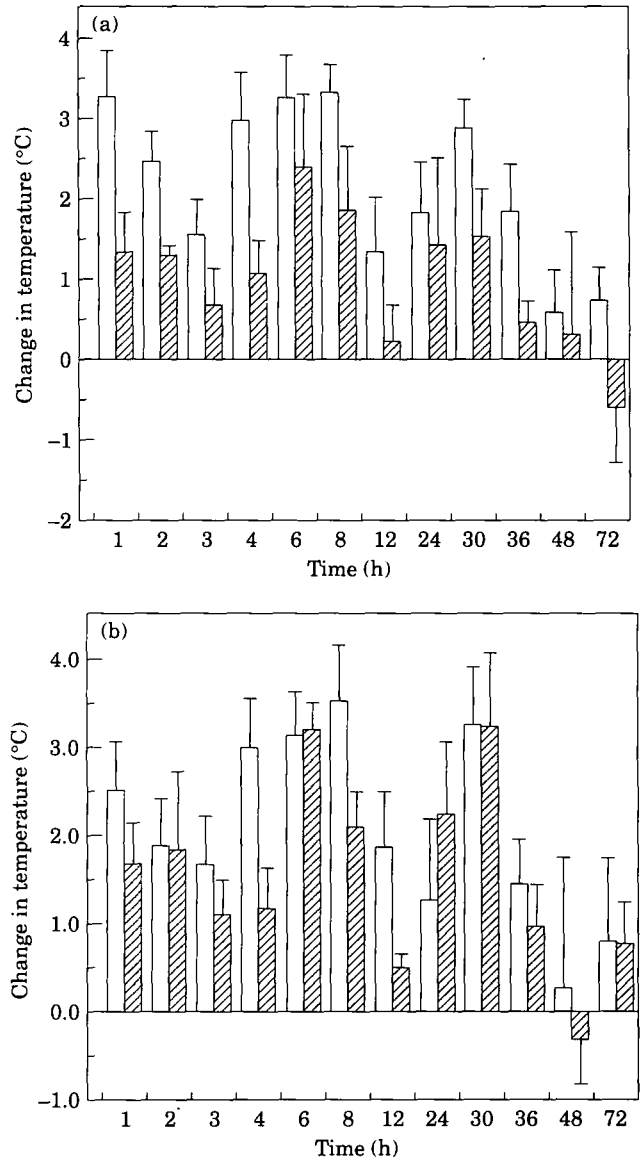

Fig. 4. Change in skin temperature from control values at sites of injection of (a) 1% dextran and (b) 4% dextran in calves that received no drug treatment (□) or carprofen (0.7 ml kg^{-1} i.v.) (▨). Carprofen and carrageenin were both injected at time 0. Each column represents the mean±SEM for six calves.

those in non-drug-treated animals (Fig. 5). The high concentration of carrageenin (0.25%) produced much larger lesions than the low concentration. In calves receiving 0.7 mg kg^{-1} carprofen, at nine out of 10 recording times lesion swellings were smaller than those obtained in the absence of drug treatment. However, the

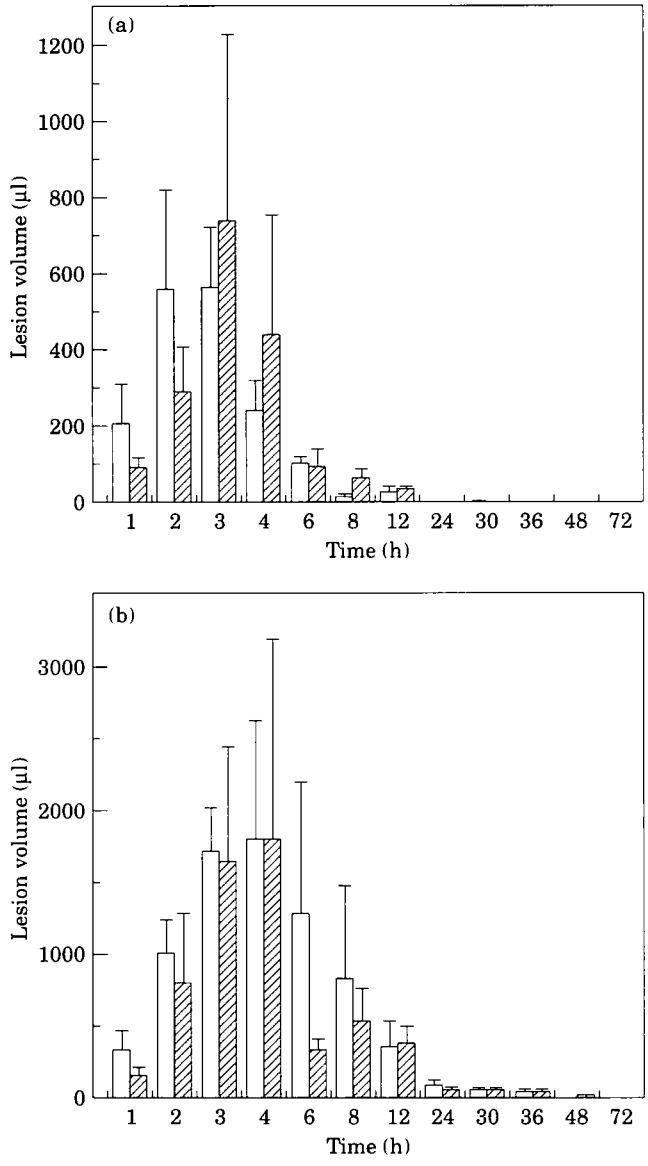

Fig. 5. Lesion volumes produced by i.d. injection of (a) 0.0625% carrageenin and (b) 0.25% carrageenin in calves that received no drug treatment (□) or carprofen (0.7 mg kg^{-1} i.v.) (▨). Carprofen and carrageenin were both injected at time 0. Each column represents the mean± SEM for six calves.

degree of attenuation of swelling was small or slight and at no time was the reduction in swelling produced by carprofen statistically significant (Fig. 5).

Relatively small and transient lesions were produced by dextran (1%) both in the absence and in the presence of carprofen and the differences were small and non-significant (data not shown). Greater, but still transient, lesion volumes were

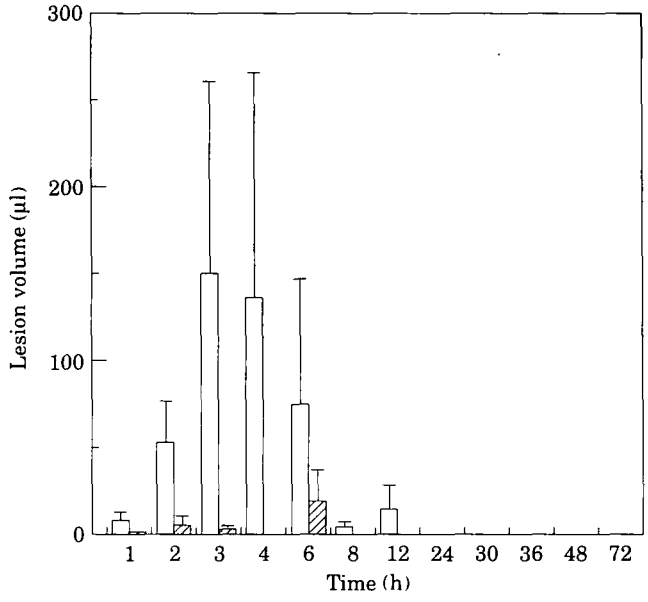

Fig. 6. Lesion volumes produced by i.d. injection of 4% dextran in calves that received no drug treatment (□) or carprofen (0.7 mg kg^{-1} i.v.) (▨). Carprofen and carrageenin were both injected at time 0. Each column represents the mean±SEM for six calves.

Table III
Percentage inhibition of *ex vivo* serum TXB_2 synthesis in calves following racemic carprofen administration (0.7 mg kg^{-1} i.v.) (mean±SEM, n=6)

Time	*Treatment group*	
	No treatment	*Carprofen*
1	−7.1±17.5	29.5±10.9
2	−12.1±32.5	9.6±15.7
3	−36.8±14.1	3.9±17.0*
4	−22.0±13.6	15.1±10.5
6	−2.6±23.6	18.2±17.5
8	25.6±16.4	54.7±12.3
12	−21.2±15.0	19.3±15.0
24	2.4±20.6	16.3±17.5
30	−3.5±12.1	49.3±14.7*
36	15.2±8.7	46.7±16.1
48	−1.6±22.6	18.5±13.5
72	18.7±24.7	26.5±20.3

The significance of differences from the control value in each group is indicated by asterisks; *P<0.05.

obtained with the higher concentration of dextran (4%). Dextran-induced swelling was virtually abolished by carprofen, although inter-animal differences in volume were large, and differences between untreated and carprofen-treated calves were not significant (Fig. 6).

Serum TXB_2 concentration

Percentage inhibition of *ex vivo* serum TXB_2 synthesis is presented in Table III. At several measuring times TXB_2 concentrations were lower following carprofen treatment compared to controls but differences were at most modest and sometimes absent. Differences from animals receiving no treatment were statistically significant only at two measuring times, 3 h and 30 h (P=0.05). The biological significance of this apparent inhibition is questionable, since the response was not time-related. Indeed, the inhibition was least (and non-significant) when it might have been expected to be greatest (between 2 and 6 h) i.e. when drug concentrations in plasma were highest.

DISCUSSION

Carprofen is a NSAID, the actions of which were first described by Randall and Baruth (1976). Studies in mice and rats revealed that it possesses analgesic, antipyretic and anti-inflammatory properties with a similar potency to indomethacin and greater activity than both phenylbutazone and aspirin. However, it was found to be 16 times less active than indomethacin in producing gastric ulcers in rats and 70 times less potent than indomethacin in blocking arachidonic acid-induced diarrhoea in mice (Randall & Baruth, 1976). Similar findings were reported by Maeda *et al.* (1977) and Strub *et al.* (1982). The latter authors reported ED_{50} values (mg kg^{-1}) for analgesia at a site of inflammation of 2.5 (carprofen), 1.2 (indomethacin), 25 (phenylbutazone) and 50 (aspirin). ED_{30} (mg kg^{-1}) values for suppression of carrageenin-induced oedema were 2 (carprofen and indomethacin), 55 (phenylbutazone) and 68 (aspirin). However, all four drugs were weakly active or inactive in non-inflammatory tests of analgesic activity (phenylquinone writhing and tail flick tests).

These early findings are of interest for two reasons. First, they indicate that carprofen may differ from most other NSAIDs in its mechanism of action. Secondly, they indicate that carprofen may have a wider safety margin than many other NSAIDs, at least in respect of gastrointestinal tolerance. Recently, Vane and Botting (1995) have reported that racemic carprofen has a low potency in inhibiting cyclo-oxygenase. Moreover, IC_{50} values for inhibition of constitutive cyclo-oxygenase (COX-1) and inducible cyclo-oxygenase (COX-2) were identical. COX-1 is believed to be concerned with a range of physiological functions, including gastro- and reno-protection, whereas COX-2 is induced at sites of inflammation and is concerned with generating inflammatory prostanoids (Appleton *et al.*, 1994). The COX-2:COX 1 inhibition ratio of 1 for carprofen is much more favourable than other commonly used NSAIDs, such as aspirin (ratio 166) and tolfenamic acid (ratio 17) (Vane & Botting, 1995).

Controlled clinical trials in three centres in the United Kingdom have shown that carprofen, administered orally at a dose rate of 4.0 mg kg^{-1} to dogs, is an effective analgesic (T. Balmer, personal communication). It controls postoperative pain with a similar efficacy to recommended doses of papaveretum and flunixin and it has also been shown to reduce lameness in geriatric dogs with osteoarthritis and similar conditions (T. Pearson, Q. A. McKellar, S. Carmichael & G.

Bell, unpublished results). Using a dose rate and route of administration of carprofen identical to those used in this study (0.7 mg kg^{-1} i.v.), Schatzmann *et al.* (1990, 1992) demonstrated an analgesic action of carprofen in horses for approximately 24 h. They concluded that plasma total carprofen concentrations in excess of 1.5 μg ml^{-1} were required to provide an analgesic effect.

The action of carprofen (0.7 mg kg^{-1} i.v.) in the horse has also been evaluated in a model of acute non-immune inflammation, based on the subcutaneous implantation of carrageenin soaked sponges in the necks of horses (Higgins & Lees, 1984). Carprofen produced only moderate decreases in serum TXB_2 and exudate PGE_2 concentrations but reduced oedema swelling to approximately half that obtained in placebo-treated horses (Lees *et al.*, 1991, 1994).

In the present investigation, the anti-inflammatory effects of carprofen were manifested as an attenuation of temperature rise at the sites of i.d. injection of the mild irritants carrageenin and dextran and as suppression of lesion swelling produced by these irritants. However, the response to carprofen was not consistent and with large inter-animal differences relatively large decreases in lesion volume failed to achieve statistical significance. On the basis of these findings, it seems improbable that cyclo-oxygenase inhibition can account solely for the anti-inflammatory properties of carprofen, since blockade of serum TXB_2 was modest and inconsistent.

Previous investigators have described pharmacokinetic parameters for carprofen in the dog (McKellar *et al.*, 1990, 1994), sheep (Welsh *et al.*, 1992), cattle (Ludwig *et al.*, 1989; Lohuis *et al.*, 1991) and horse (Graser *et al.*, 1991; Lees *et al.*, 1994). However, with the exception of two preliminary reports (Graser *et al.*, 1991; Lees *et al.*, 1991) and two recent articles (Delatour *et al.*, 1993; McKellar *et al.*, 1994), these authors measured only total concentrations of carprofen in plasma, and there are no reports describing the pharmacokinetics of carprofen enantiomers in cattle. This study has demonstrated numerically small to moderate but significant differences in the pharmacokinetics of the R(−) and S(+) enantiomers of carprofen in calves. Thus $t_{\frac{1}{2}\beta}$ and MRT were longer, Cl slower and AUC greater for the R(−) enantiomer. Volumes of distribution (Vd_{area} and Vd_{ss}) were less for the R(−) compared to the S(+) enantiomer and these differences were also statistically significant though numerically small. Since drug distribution is generally dependent primarily on physico-chemical properties, in particular lipid solubility, which would normally be similar for two enantiomers, the relatively similar distribution volumes reported for R(−) and S(+) carprofen are not surprising. The small differences that did occur are not explained by the present findings but they might be due to differences in binding to plasma protein.

Whether differences in AUC, Cp(48h), MRT, Cl and $t_{\frac{1}{2}\beta}$ for R(−) and S(+) carprofen in the calf reflect enantiomeric differences in metabolism and/or excretion or whether they are due to chiral inversion of S(+) to R(−) carprofen is not known. However, the latter seems unlikely, since chiral inversion of carprofen (in either direction) has been shown not to occur in either the horse (P. Delatour & P. Lees, unpublished results) or dog (McKellar *et al.*, 1994). Moreover, when chiral inversion of arylpropionic acids does occur, it usually involves conversion of the R(−) to the S(+) enantiomer (Caldwell *et al.*, 1988; Evans, 1992). It is thus likely that altered metabolism and/or excretion explains the differences

in pharmacokinetics between R(−) and S(+) carprofen in calves and it is interesting to note that qualitatively similar but quantitatively greater differences have been described for the horse and dog. Thus, the plasma concentration-time AUC ratio (R:S) following i.v. administration of racemic carprofen (0.7 mg kg^{-1}) to horses was 82:18 and in the dog oral dosing of racemic carprofen (4.0 mg kg^{-1}) produced an R:S ratio of 62:38. The AUC ratio (R:S) in this study was 58:42. It was recently demonstrated in dogs and horses (Soraci *et al.*, 1995) that the plasma chiral behaviour of carprofen was a consequence of its liver enantioselective glucuronidation.

Enantiomeric differences in the pharmacokinetics of chiral drugs are highly significant for therapeutic efficacy, since biological activity commonly resides in a single enantiomer and high eudismic ratios are the rule rather than the exception (Williams & Lee, 1985). Many studies in laboratory animals have demonstrated that at molecular (inhibition of cyclo-oxygenase) and whole animal (suppression of inflammatory swelling) levels the eudismic ratio for 2-arylpropionic acid NSAIDs, S:R, is commonly 20:1 or greater. Therefore, it is commonly assumed that in the clinical patient R(−) enantiomers such as R(−)-carprofen contribute little to the therapeutic and toxic effects of NSAIDs. However, inhibition of prostaglandin synthesis and anti-inflammatory activity do not always run in parallel. Thus, the potency ratios, S:R, for inhibition of prostaglandin synthesis and attenuation of carrageenin-induced paw oedema are 1000:1 and 10:1, respectively, for the NSAID clidanac but the enantiomers of this agent are approximately equipotent in inhibiting erythema, induced by UV light, in the guinea pig (Kuzuma *et al.*, 1974; Tamura *et al.*, 1981). Similarly, the potency ratios for ibuprofen (S:R) are 165:1 for inhibition of prostaglandin synthesis, 1.4:1.0 for inhibition of oedematous swelling and 1.1:1.0 for blockade of UV-induced erythema (Adams *et al.*, 1976). Some of these differences, with ibuprofen for example, may be due to *in vivo* metabolic chiral inversion and this phenomenon clearly complicates attempts to determine potency ratios of drug enantiomers *in vivo*.

Recently, Brune *et al.* (1992) demonstrated that R-flurbiprofen, which is not inverted in humans or rats, is devoid of activity against cyclo-oxygenase and is non-toxic to the gastrointestinal tract. Moreover, it is only weakly active as an anti-oedematous agent. However, it is an effective analgesic and these authors conclude that R-flurbiprofen and possibly other enantiomers of 2-arylpropionic acids may exert analgesic effects independently of prostaglandin synthesis inhibition (McCormack & Brune, 1991). Hence, it is possible that both enantiomers of carprofen may contribute to the drug's therapeutic effects, but further studies with the separate enantiomers in models of inflammation and pain will be required to elucidate such activity. The feasibility of conducting such studies is favoured by the likelihood that chiral inversion of carprofen enantiomers does not occur.

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REFERENCES

ADAMS, S. S., BRESFOFF, P. & MASON, C. G. (1976). Pharmacological differences between the optical isomers of ibuprofen: evidence for metabolic inversion of the (−) isomer. *Journal of Pharmacy and Pharmacology* **28,** 256–7.

APPLETON, I., TOMLINSON, A. & WILLOUGHBY, D. A. (1994). Inducible cyclo-oxygenase (COX-2): a safer therapeutic target? *British Journal of Rheumatology* **33,** 410–92.

BRUNE, K., GEISSLINGER, G. & MENZEL-SOGLOWCK, S. L. (1992). Pure enantiomers of 2-arylpropionic acids: tools in pain research and improved drugs in rheumatology. *Journal of Clinical Pharmacology* **32,** 944–52.

CALDWELL, J., HUTT, A. J. & FOURNEL-GIGLEUX, S. (1988). The metabolic chiral inversion and dispositional enantioselectivity of the 2-arylpropionic acids and their biological consequences. *Biochemical Pharmacology* **37,** 105–14.

DELATOUR, P., BENOIT, E., BOURDIN, M., GOBRON, M. & MOYSAN, F. (1993). Enantiosélectivé comparée de la dispositon de deux anti-inflammatoires non steroïdiens, le kétoprofène et le carprofène, chez l'homme et l'animal. *Bulletin Académie Nationale de Médecine* **177,** 515–27.

DELATOUR, P., BENOIT, E. & SORACI, A. (1994). Drug chirality: its significance in veterinary pharmacology and therapeutics. In *Proceedings of the 6th International Congress of the European Association for Veterinary Pharmacology and Toxicology*, pp. 6–9. Oxford: Blackwell Scientific Publications.

EVANS, A. M. (1992). Enantioselective pharmacodynamics and pharmacokinetics of chiral non-steroidal anti-inflammatory drugs. *European Journal of Clinical Pharmacology* **42,** 237–56.

FOSTER, R. T. & JAMALI, F. (1987). High performance liquid chromatography assay of ketoprofen enantiomers in human plasma and urine. *Journal of Chromatography* **416,** 388–93.

GAUT, Z. W., BARUTH, L., RANDALL, L. O., ASHLEY, C. & PAULSRUD, J. R. (1975). Stereoisomeric relationships among anti-inflammatory activity, inhibition of platelet aggregation and inhibition of prostaglandin synthetase. *Prostaglandins* **10,** 59–66.

GRASER, T. A., JORDAN, J.-C., KARPF, M. & HOLCK, M. (1991). Determination of carprofen enantiomers: application to biological fluids of target species. *Acta Veterinaria Scandinavica Supplement* **74,** 247–8.

HIGGINS, A. J. & LEES, P. (1984). Arachidonic acid metabolites in carrageenin-induced equine inflammatory exudate. *Journal of Veterinary Pharmacology and Therapeutics* **7,** 65–72.

KUZUMA, S., MATSUMOTO, N., KOMETANI, T. & KAWAI, K. (1974). Biological activities of optical isomers of 6-chloro-5-cyclohexylindan-1-carboxylic acid (TAI-248:anti-inflammatory agents). *Japanese Journal of Pharmacology* **24,** 695–705.

LEES, P., EWINS, C. P. TAYLOR, J. B. O. & SEDGWICK, A. D. (1987). Serum thromboxane in the horse and its inhibition by aspirin, phenylbutazone and flunixin. *British Veterinary Journal* **143,** 462–76.

LEES, P., DELATOUR, P., BENOIT, E. & FOSTER, A. P. (1991). Pharmacokinetics of carprofen enantiomers in the horse. *Acta Veterinaria Scandinavica Supplement* **87,** 249–51.

LEES, P., MCKELLAR, Q. A., MAY, S. A. & LUDWIG, B. M. (1994). Pharmacodynamics and pharmacokinetics of carprofen in the horse. *Equine Veterinary Journal* **26,** 203–8.

LOHUIS, J. A. C. M., VAN WERVEN, T., BRAND, A. *et al.* (1991). Pharmacodynamics and pharmacokinetics of carprofen, a non-steroidal anti-inflammatory drug in healthy cows and cows with *Escherichia coli* endotoxin-induced mastitis. *Journal of Veterinary Pharmacology and Therapeutics* **14,** 219–29.

LUDWIG, B. M., JORDAN, J-C., REKUN, W. F. & THUN, R. (1989). Carprofen in veterinary medicine. 1. Plasma disposition, milk excretion and tolerance of carprofen in milk producing cows. *Schweizer Archiv für Tierheilkunde* **131,** 99–106.

MAEDA, M., TANOKA, Y., SUSUKI, T. & NAKAMURA, K. (1977). Pharmacological studies on carprofen a new NSAID in animals. *Folia Pharmacologica Japonica* **73,** 757–77.

MCCORMACK, K. & BRUNE, K. (1991). Dissociation between the anti-nociceptive and anti-inflammatory effects of the non-steroidal anti-inflammatory drugs. A survey of their analgesic efficiency. *Drugs* **41,** 533–47.

McKellar, Q. A., Pearson, T., Bogan, J. A. *et al.* (1990). Pharmacokinetics, tolerance and serum thromboxane inhibition of carprofen in the dog. *Journal of Small Animal Practice* **31,** 443–8.

McKellar, Q. A., Delatour, P. & Lees, P. (1994). Stereospecific pharmacodynamics and pharmacokinetics of carprofen in the dog. *Journal of Veterinary Pharmacology and Therapeutics* **17,** 447–54.

Randall, L. O. & Baruth, H. (1976). Analgesic and anti-inflammatory activity of 6-chloro-alpha-methyl-carbazole-2-acetic acid (C-5720). *Archives Internationale Pharmacodynamie* **220,** 94–114.

Schatzmann, U., Gugelmann, M., Von Cranach, J., Ludwig, B. M. & Rehm, W. F. (1990). Pharmacodynamic evaluation of the peripheral pain inhibition by carprofen and flunixin in the horse. *Schweizer Archiv Für Tierheilkunde* **132,** 497–504.

Schatzmann, U., Gugelmann, M., Cranach, J. *et al.* (1992). Visceral and peripheral pain detection models in the horse using flunixin and carprofen. In *Animal Pain*, eds C. E. Short & A. V. Poznak, pp. 411–20. Edinburgh: Churchill Livingston Inc.

Soraci, A., Benoit, E., Jaussaud, P., Lees, P. & Delatour, P. (1995). Enantioselective glucuronidation and subsequent biliary excretion of carprofen in horses. *American Journal of Veterinary Research* **56,** (in press).

Strub, K. M., Aeppli, L. & Müller, R. K. M. (1982). Pharmacological properties of carprofen. *European Journal of Rheumatology and Inflammation* **5,** 478–87.

Tamura, S., Kujuna, S. & Kawai, K. (1981). Inhibition of prostaglandin biosynthesis by clidanac and related compounds: structural and conformational requirements for PG synthetase inhibition. *Journal of Pharmacy and Pharmacology* **33,** 29–32.

Vane, J. R. & Botting, R. M. (1995). New insights into the mode of action of anti-inflammatory drugs. *Inflammation Research* **44,** 1–10.

Welsh, E. M., Baxter, P. & Nolan, A. M. (1992). Pharmacokinetics of carprofen administered intravenously to sheep. *Research in Veterinary Science* **53,** 264–6.

Williams, K. & Lee, E. C. (1985). Importance of drug enantiomers in clinical pharmacology. *Drugs* **30,** 333–54.

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