# EVALUATION OF CARPROFEN IN CALVES USING A TISSUE CAGE MODEL OF INFLAMMATION

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## SUMMARY

The arylpropionate anti-inflammatory drug, carprofen, was administered intravenously as the racemate at a dose rate of 0.7 mg $kg^{-1}$ body weight to six Friesian bull calves aged 16–17 weeks. Anti-inflammatory and pharmacokinetic properties were investigated using a tissue cage model of inflammation based on intracaveal injection of the mild irritant, carrageenin. Carprofen displayed enantioselective pharmacokinetics, with the R(−) enantiomer predominating in plasma at all measuring times. Elimination half-life and mean residence time were shorter and volume of distribution and clearance were greater for the S(+) than for the R(−) enantiomer. Penetration of both enantiomers into transudate (non-stimulated tissue cage) was poor but penetration into exudate (carrageenin-stimulated tissue cage) was good. Carprofen failed to reduce exudate concentration of prostaglandin $E_2$ and the reductions in 12-hydroxyeicosatetraenoic acid were non-significant at most sampling times. The long elimination half-life of both R(−) and S(+) carprofen enantiomers and their ready penetration into and slow clearance from inflammatory exudate indicate that the drug is likely to have a long duration of action in calves. The mechanism of action is unknown but it is unlikely to involve inhibition of either cyclo-oxygenase or 12-lipoxygenase.

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

## INTRODUCTION

Carprofen is a 2-arylpropionic acid derivative that has proved to be a relatively weak inhibitor of cyclo-oxygenase in laboratory animal studies (Strub *et al.*, 1982),

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in the dog (McKellar *et al.*, 1990, 1994) and in the horse (Lees *et al.*, 1994). The latter authors showed that a dose rate of 0.7 mg $kg^{-1}$ administered intravenously to horses reduced lesion swelling in a model of acute soft tissue inflammation to approximately half the volume recorded in placebo-treated animals, but exudate prostaglandin (PG)$E_2$ and serum thromboxane (TX)$B_2$ concentrations were reduced only moderately. It is therefore likely that carprofen acts, at least partially, by mechanisms other than inhibition of synthesis of inflammatory prostanoids such as $PGE_2$. This may be advantageous when comparing carprofen with other non-steroidal anti-inflammatory drugs (NSAIDs), such as phenylbutazone and flunixin, in widespread use in veterinary medicine. These drugs, at recommended dose rates, are potent cyclo-oxygenase inhibitors in horses (both drugs) or horses and calves (phenylbutazone) (Higgins *et al.*, 1984a, 1987; Lees & Higgins, 1984; Lees *et al.*, 1987). Since cyclo-oxygenase inhibition is believed to underlie the toxic effects of these agents on the gastrointestinal tract, liver and kidney, an agent such as carprofen, which acts principally or solely by other mechanisms, might possess a wider margin of safety in clinical use.

The development of a tissue cage model of acute inflammation by Higgins *et al.* (1984b) has facilitated studies of the pharmacodynamics and pharmacokinetics of NSAIDs in horses, calves and dogs (Lees & Higgins, 1984; Higgins *et al.*, 1987; McKellar *et al.*, 1994; Landoni *et al.*, 1995; Landoni & Lees, 1995). In this investigation this model has been used, in association with conventional pharmacokinetic approaches:

(1) to determine plasma concentration-time relationships and establish pharmacokinetic parameters for carprofen enantiomers in 16–17 week old calves and to compare the data with the findings of a previous study in 8–10 week old calves (Delatour *et al.*, 1996) at a dose rate of 0.7 mg $kg^{-1}$ of the racemic mixture administered intravenously.
(2) to measure the penetration into (a) tissue cage fluid (transudate) and (b) inflammatory exudate of carprofen enantiomers;
(3) to establish the magnitude and time-course of inhibition of exudate $PGE_2$ and 12-hydroxyeicosatetraenoic acid (12-HETE) synthesis, as markers of exudate cyclo-oxygenase and 12-lipoxygenase activity, respectively;
(4) to determine the magnitude and time course of inhibition by carprofen of the oedematous swelling induced by intradermal (i.d.) injection of the mild irritant, zymosan.

## MATERIALS AND METHODS

### *Animals and experimental design*

A cross-over experimental design in six healthy Friesian bull calves, aged 16–17 weeks and weighing 110–129 kg, was used. Tissue cages, four per animal at subcutaneous sites, on the left flank, were inserted under deep xylazine (Rompun, Bayer plc) sedation and local anaesthesia using 2% (w/v) lignocaine with adrenaline (Norbrook Laboratories Ltd). The animals were allowed to recover from surgery for 23 days, and this permitted the growth of granulation tissue into the cages (Higgins *et al.*, 1984b).

In part 1 of the study, at time 0, three calves received 0.7 mg $kg^{-1}$ carprofen

(Zenecarp Injection, Grampian Pharmaceuticals Ltd) intravenously and three received no treatment (controls). A 7-day interval was allowed between the two parts of the cross-over. In part 2, treatments were reversed. Also at time 0, each of the six calves received an intracaveal (i.c.) injection of the mild irritant carrageenin; 0.5 ml of 1% (w/v) sterile solution injected into a single tissue cage. The three non-injected tissue cages were used to collect, in rotation, tissue cage fluid (transudate) at pre-determined times, whilst the carrageenin injected cage was used to harvest inflammatory exudate. Also at time 0, intradermal (i.d.) injections (three per calf) of 0.1 ml volumes of sterile aqueous solutions of zymosan in saline (1%, 0.25% and 0.0625%, w/v) were made into clipped and shaved areas on one side of the neck. The left neck was used for zymosan injections in part 1 and the right neck was used in part 2 of the cross-over. Lesion swelling (volume) at i.d. sites of zymosan injection were monitored at pre-determined times, as previously described (Delatour *et al.*, 1996).

*Blood, exudate and transudate samples*

Samples of blood (for determination of plasma carprofen enantiomer concentrations and circulating leucocyte numbers), inflammatory exudate (for determination of carprofen enantiomer and protein concentrations, leucocyte count and concentrations of the eicosanoids $PGE_2$ and 12-HETE) and transudate (for determination of carprofen enantiomer concentrations, protein concentration and leucocyte numbers) were collected at pre-determined times. All samples were placed on ice prior to centrifugation. Blood samples (10 ml) for measurement of plasma carprofen concentration were collected in Li-heparin monovettes (Sarstedt Ltd). K-EDTA tubes obtained from Sarstedt Ltd were used to collect blood samples (5 ml) for determination of leucocyte counts. Exudate and transudate were collected in tubes containing 10 μg BW 540C (Glaxo Wellcome plc), a dual cyclo-oxygenase lipoxygenase inhibitor, incorporated to prevent the artefactual generation of eicosanoids *in vitro*. To each tube 50 IU heparin were also added. All plasma, exudate and transudate samples were stored at −20°C prior to analysis. Prior to freezing, exudate and transudate samples were centrifuged within 30 min of collection for 10 min at 4°C and 2500 ***g*** and the supernatant removed. $PGE_2$ concentration in exudate was measured by radio-immunoassay as previously described (Higgins & Lees, 1984a). 12-HETE concentration in exudate was also measured by radio-immunoassay ([$^3$H] RIA Kit: Advanced Magnetics Inc.). Cell counts were determined within 3 h of sample collection using a Coulter Counter (Model ZM) and protein concentrations were measured using the Biuret reaction (Model Gilford SPA 300). Lesion volumes and carprofen concentrations in plasma, exudate and transudate were measured as described previously (Delatour *et al.*, 1996). Pharmacokinetic constants were also calculated as described by Delatour *et al.* (1996).

## RESULTS

*Pharmacokinetics of carprofen and its enantiomers*

The calves used in this investigation were almost twice as old (16–17 weeks) and

weighed almost twice as much (116–119 kg) as those used in a previous investigation of carprofen pharmacokinetics (Delatour *et al.*, 1996). Plasma concentration data are presented in Table I. Some pharmacokinetic parameters (for definitions, see Table II) were similar in the two groups of calves (A, B, $\alpha$, $t_{\frac{1}{2}\alpha}$, Vc, $Vd_{area}$ and $Vd_{ss}$). However, there were statistically significant differences for other parameters [$\beta$, $t_{\frac{1}{2}\beta}$, MRT, Cl and Cp(48h)]. Elimination half-life was significantly shorter in the older calves for total drug (43.4 *vs* 33.8 h), R(−) enantiomer (49.7 *vs* 37.7 h) and S(+) enantiomer (37.4 *vs* 29.1 h). Mean residence times and plasma drug concentrations at 48 h were also shorter for both enantiomers in older calves (Table II).

Enantioselective pharmacokinetics of carprofen was previously reported in 8–10 week old calves (Delatour *et al.*, 1996). Differences in pharmacokinetic variables between (R−) and S(+) enantiomers were again apparent in the older calves used in this investigation (Table II). The differences were for all pharmacokinetic parameters except A (zero time intercept, distribution phase) and volume of the central compartment, statistically significant. Thus, $t_{\frac{1}{2}\alpha}$ and $t_{\frac{1}{2}\beta}$ were shorter for the S(+) carprofen enantiomer, whilst $Vd_{area}$ and $Vd_{ss}$ were smaller for the R(−) enantiomer. MRT was longer and Cl was slower for the R(−) enantiomer and Cp(48h) and $AUC_{0-144}$ were both greater for the R(−) enantiomer.

*Distribution of carprofen into transudate and exudate*

The relatively low values for $Vd_{area}$ and $Vd_{ss}$ for total carprofen (147 and 146 ml $kg^{-1}$, respectively) indicate that the drug is not widely distributed in the body, probably as a consequence of a high degree of binding to plasma proteins. This may explain the relatively poor penetration of carprofen into transudate

**Table I**
**Plasma carprofen concentrations ($\mu g\ ml^{-1}$) in calves following i.v. dosing of racemic carprofen at 0.7 mg $kg^{-1}$ (mean±SEM, $n$=5)**

| *Time (h)* | *Total carprofen* | *R(−) carprofen* | *S(+) carprofen* |
|---|---|---|---|
| 0.083 | 10.98±0.83 | 5.66±0.42 | 5.32±0.41 |
| 1.0 | 6.44±0.49 | 3.50±0.25 | 2.94±0.25 |
| 1.5 | 5.40±0.48 | 2.99±0.25 | 2.50±0.23 |
| 2.0 | 5.41±0.32 | 3.02±0.17 | 2.39±0.17 |
| 3.0 | 5.15±0.35 | 2.81±0.20 | 2.34±0.15 |
| 4.0 | 4.81±0.19 | 2.60±0.12 | 2.21±0.07 |
| 6.0 | 4.22±0.10 | 2.28±0.06 | 1.94±0.04 |
| 9.0 | 3.59±0.18 | 1.92±0.10 | 1.67±0.08 |
| 12.0 | 3.30±0.20 | 1.79±0.11 | 1.51±0.09 |
| 24.0 | 2.66±0.10 | 1.49±0.05 | 1.18±0.05 |
| 30.0 | 2.33±0.09 | 1.33±0.05 | 1.00±0.05 |
| 36.0 | 1.86±0.11 | 1.08±0.06 | 0.78±0.05 |
| 48.0 | 1.62±0.07 | 0.95±0.03 | 0.67±0.04 |
| 96.0 | 0.75±0.08 | 0.50±0.05 | 0.26±0.03 |

Weight=115.7±2.2 kg (part 1); 119.2±2.8 kg (part 2).
Age=16 weeks (part 1); 17 weeks (part 2).

**Table II**
**Pharmacokinetic variables following racemic carprofen administration (0.7 mg kg$^{-1}$ i.v.) (mean±SEM, $n$=5).**

| *Variable* | *Total carprofen* | *R(−) carprofen* | *S(+) carprofen* |
|---|---|---|---|
| A (μg ml$^{-1}$) | 6.82±0.75 | 3.37±0.38 | 3.41±0.36* |
| B (μg ml$^{-1}$) | 4.66±0.13 | 2.49±0.06 | 2.22±0.09†† |
| α (h$^{-1}$) | 1.37±0.18 | 1.19±0.15 | 1.59±0.24 |
| β (h$^{-1}$) | 0.021±0.001* | 0.019±0.001* | 0.024±0.001*,†† |
| $t_{\frac{1}{2}\alpha}$ (h) | 0.546±0.081 | 0.624±0.089 | 0.476±0.067 |
| $t_{\frac{1}{2}\beta}$ (h) | 33.8±1.7* | 37.7±1.8* | 29.1±1.6*,†† |
| Vc (ml kg$^{-1}$) | 62.4±5.3 | 61.3±5.2 | 63.6±5.3 |
| $Vd_{area}$ (ml kg$^{-1}$) | 147.0±4.4 | 136.2±2.9 | 154.4±5.4†† |
| $Vd_{ss}$ (ml kg$^{-1}$) | 146.0±5.0 | 140.2±4.2 | 154.0±7.5† |
| MRT (h) | 49.4±3.2* | 56.0±3.6* | 41.9±2.8*,†† |
| Cl (ml h$^{-1}$ kg$^{-1}$) | 2.984±0.094 | 2.524±0.098* | 3.696±0.113*,††† |
| Cp(48h) (μg ml$^{-1}$) | 1.62±0.07* | 0.95±0.03* | 0.67±0.04*,††† |

*$P$<0.05, comparison of young calves (Delatour *et al.*, 1996) and older calves (this investigation).

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

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

(Fig. 1). Thus, total mean carprofen concentrations (μg ml$^{-1}$) in plasma and transudate were: 5.41 and 0.43 at 2 h, 3.30 and 1.12 at 12 h and 1.86 and 1.25 at 36 h, respectively. Only at 96 h did the mean transudate concentration (0.90 μg ml$^{-1}$) exceed the plasma concentration (0.75 μg ml$^{-1}$). On the other hand, carprofen penetrated much more readily into inflammatory exudate. Thus, mean plasma, exudate and transudate concentrations (μg ml$^{-1}$) of total carprofen were 4.81, 2.10, 0.65 (4 h), 3.59, 3.78, 0.82 (9 h), 2.66, 3.50, 1.44 (24 h) and 1.62, 2.16, 1.28 (48 h), respectively. Exudate carprofen concentration first exceeded plasma concentration at approximately 9 h and thereafter concentrations were greater in exudate through to the final sampling time of 96 h (Fig. 1). Drug penetration into exudate occurred relatively rapidly; peak concentration was recorded in most animals at 9–12 h. On the other hand, peak concentrations of carprofen in transudate were not obtained until 24–48 h.

In spite of the differing total concentrations of carprofen in the three fluids, plasma, exudate and transudate, the enantiomer concentrations expressed in percentage terms were very similar, indeed almost identical, in each fluid at each sampling time (Table III).

*Lesion volume*

With the lowest zymosan concentration (0.0625%) administered intradermally peak swelling occurred at 3 h. In carprofen-treated calves, lesion volumes were

smaller at all recording times up to and including 24 h. The reduction in volume at most recording times from 0–24 h approached or exceeded 40% but differences were not statistically significant (Fig. 2).

The intermediate and high zymosan concentrations produced two swelling peaks, at 3 and 12 h (Fig. 2). The magnitude of the swelling was smaller in carprofen treated calves between 3 and 12 h. Thereafter, lesion volumes were greater in drug-treated calves. However, the increase with the intermediate zymosan concentration was attributable wholly to an untypically marked and delayed but persistent response in a single animal (calf X). None of the differences in lesion volume between untreated and carprofen-treated calves were statistically significant at any time point.

*Exudate eicosanoid concentrations*

In non-drug-treated calves, exudate $PGE_2$ was initially (2 h sample) below the level of detection of the radio-immunoassay. Measurable concentrations were

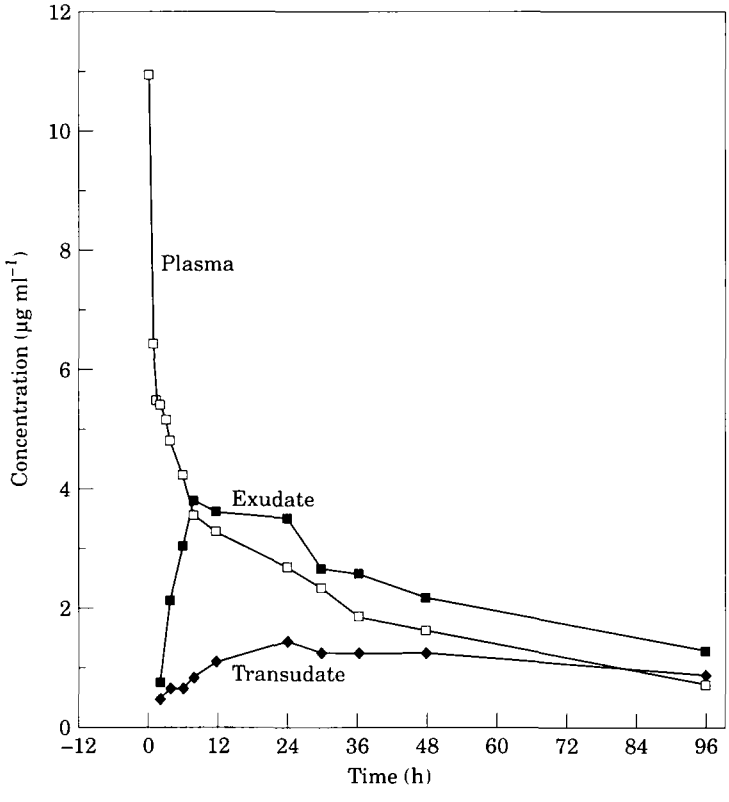


**Fig. 1.** Plasma, exudate and transudate concentrations of total carprofen (μg $ml^{-1}$) following i.v. administration of racemic carprofen (0.7 mg $kg^{-1}$). Carprofen and carrageenin both injected at zero time. Each point represents the mean±SEM for four calves (exudate and transudate) and five calves (plasma).

**Table III**

**Plasma transudate and exudate concentrations of carprofen enantiomers (% of total concentration) following i.v. racemic carprofen dosing (0.7 mg $kg^{-1}$) and i.c. carrageenin administration in calves (mean±SEM; $n$=5 plasma, $n$=4 transudate and exudate)**

| *Time (h)* | *Plasma* | | *Transudate* | | *Exudate* | |
|---|---|---|---|---|---|---|
| | *R(−) carprofen* % | *S(+) carprofen* % | *R(−) carprofen* % | *S(+) carprofen* % | *R(−) carprofen* % | *S(+) carprofen* % |
| 2 | 55.8±0.8 | 44.2±0.8 | 57.3±1.6 | 42.7±1.6 | 57.1±3.2 | 42.9±3.2 |
| 4 | 54.0±0.4 | 46.0±0.4 | 56.2±2.8 | 43.9±2.8 | 54.5±0.2 | 45.5±0.2 |
| 6 | 53.9±0.2 | 46.0±0.2 | 56.0±2.5 | 43.9±2.5 | 54.6±0.7 | 45.4±0.7 |
| 9 | 53.5±0.5 | 46.5±0.5 | 53.6±1.0 | 46.5±1.0 | 53.1±1.0 | 46.9±1.0 |
| 12 | 54.1±0.4 | 45.8±0.5 | 53.3±1.3 | 46.7±1.2 | 54.4±0.6 | 45.6±0.6 |
| 24 | 55.8±0.4 | 44.1±0.4 | 53.5±0.7 | 46.5±0.7 | 54.5±0.3 | 45.5±0.3 |
| 30 | 56.3±0.7 | 43.0±0.5 | 55.6±0.6 | 44.4±0.6 | 55.9±0.7 | 44.0±0.7 |
| 36 | 58.0±0.2 | 42.0±0.2 | 56.1±0.4 | 43.9±0.4 | 56.8±0.3 | 43.1±0.4 |
| 48 | 58.9±0.8 | 41.1±0.8 | 57.8±0.4 | 42.2±0.4 | 58.0±0.8 | 42.0±0.8 |
| 96 | 66.0±1.5 | 33.9±1.6 | 63.6±2.7 | 36.4±2.7 | 62.6±2.4 | 37.4±2.4 |

present in most samples at and beyond 4 h, with a mean peak concentration of 22.5 ng $ml^{-1}$ occurring at 9 h. By 96 h, the mean concentration had decreased to 1.2 ng $ml^{-1}$ (Fig. 3). In carprofen-treated calves, mean concentrations followed a broadly similar time course, with a peak concentration of 20.4 ng $ml^{-1}$ occurring at 9 h. There was no inhibition of $PGE_2$ synthesis at any sampling time.

In non-drug-treated animals, two 12-HETE concentration peaks occurred, at 6 and 96 h. Carprofen treatment reduced 12-HETE concentrations at nine out of 10 sampling times, indicating possible partial inhibition of 12-lipoxygenase. However, 12-HETE production was not abolished at any time, and differences between drug-treated and untreated calves achieved statistical significance at only one time (96 h, $P<0.05$).

*Exudate and transudate protein concentrations*

Initially, at 2 h, the mean protein concentration in exudate was 39 g $l^{-1}$, which is approximately 50% of normal plasma protein concentration. In non-drug-treated calves protein concentration subsequently rose, the maximum value occurring at 24 h, when a mean concentration of 56 g $l^{-1}$ was obtained (data not shown). In carprofen-treated calves, similar increases in protein concentration occurred to a maximum of 55 g $l^{-1}$, again at 24 h. At no time point did protein concentrations differ significantly in non-treated and drug-treated calves.

Protein concentration in transudate was identical to the initial (2 h) exudate concentration, with mean values of 39 g $l^{-1}$ in both non-drug-treated and carprofen-treated calves. Mean concentrations of protein in transudate showed little tendency to change with time and there were no differences between non-treated and drug-treated calves at any time point (data not shown).

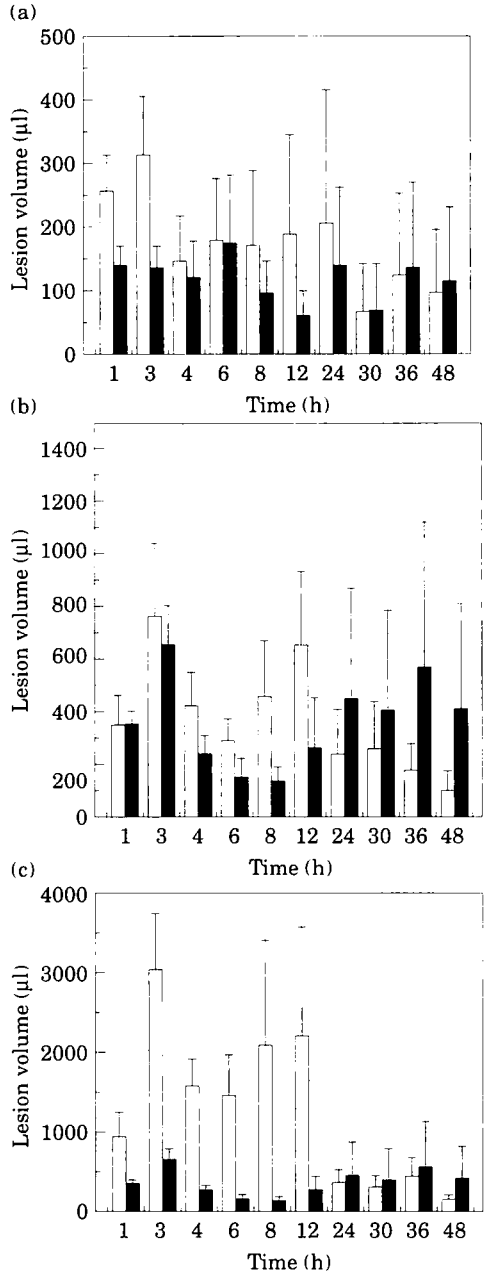


**Fig. 2.** Lesion volumes produced by i.d. injection of (a) 0.0625% zymosan, (b) 0.25% zymosan and (c) 1% zymosan in calves which received no drug treatment (□) or carprofen (■) (0.7 mg $kg^{-1}$ i.v.). Carprofen and zymosan were both injected at zero time. Each column represents the mean±SEM for six calves.

*Leucocyte count in whole blood, exudate and transudate*

Leucocyte numbers in whole blood increased in non-drug-treated calves from a

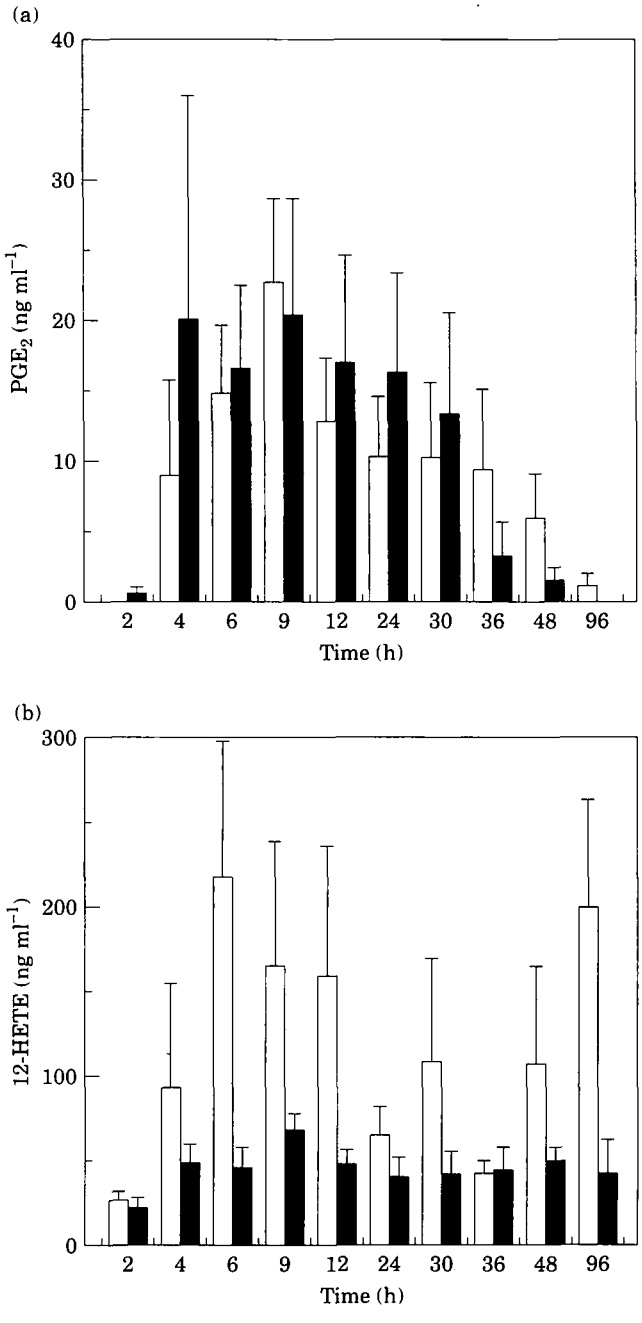


**Fig. 3.** Concentrations of (a) exudate $PGE_2$ and (b) exudate 12-HETE in calves that received no drug treatment (□) or carprofen (■) (0.7 mg $kg^{-1}$ i.v.). Carprofen and carrageenin were both injected at zero time. Each column represents the mean±SEM for six calves.

control value of $8.0\times10^9\ l^{-1}$ to $11.9\times10^9\ l^{-1}$ at 6 h. Circulating leucocyte numbers were increased for 30 h but had returned to normal by 36 h. Similar changes occurred in carprofen-treated calves. There were no differences in circulating cell numbers between the two treatment groups at any sampling time (data not shown).

Cell numbers in inflammatory exudate rose to a mean peak of $18.5\times10^9\ l^{-1}$ at 24 h from $8.4\times10^9\ l^{-1}$ at 4 h. In drug-treated animals leucocyte numbers were similar to those in non-treated animals and differences between untreated and carprofen-treated calves were non-significant at all times.

Small numbers of cells were present in transudate at all sampling times but showed little tendency to change with time. There were no apparent differences between the two groups at any sampling time (data not shown).

## DISCUSSION

The question of how carprofen acts is still unresolved. Gaut *et al.* (1975) showed that carprofen is a weak cyclo-oxygenase inhibitor, with the R(−) enantiomer being much less potent (eudismic ratio, S:R, 160:1). It possess some activity against phospholipase $A_2$ (Hope & Walton, 1983) but it is unlikely that this explains the therapeutic effects, since the synthesis of eicosanoids produced by both arms of the arachidonic acid pathway, $PGE_2$ and 12-HETE in inflammatory exudate, was not inhibited in the present investigation in calves nor in similar studies in dogs (McKellar *et al.*, 1990, 1994). Baruth *et al.* (1986) further demonstrated that carprofen did not affect 5-lipoxygenase and therefore did not inhibit leukotriene (LT) generation. In the present investigation, carprofen tended to reduce inflammatory exudate concentrations of the 12-lipoxygenase product, 12-HETE. However, this finding is of questionable significance, since 12-HETE synthesis was not abolished at any sampling time and the reduction was statistically significant only at the final sampling time of 96 h. The present findings therefore provided no evidence of significant activity of carprofen against inflammatory cell cyclo-oxygenase or 12-lipoxygenase.

McKenzie (1994) recently described interesting actions of several newer carboxylic acid NSAIDs that inhibit cyclo-oxygenase but only at high dose rates. One compound, IX 207-987, suppressed adjuvant-induced arthritis and *Escherichia coli*-induced fever but it was not ulcerogenic. IX 207-987 inhibited both interleukin (IL)-1α and IL-1β secretion by human mononuclear cells at concentrations achieved *in vivo*. A recent report by Villaneuva *et al.* (1993) on ibuprofen and its enantiomers provides further insights into possible mechanisms of action of arylpropionate NSAIDs. These authors showed that rac-ibuprofen inhibited a number of human leucocyte functions (superoxide generation, β-glucuronidase release and $LTB_4$ formation) and similar $IC_{50}$ values were obtained for the S(+) and R(−) enantiomers. Moreover, these effects were independent of cyclo-oxygenase inhibition. It will be of interest to determine whether carprofen shares any of these actions of ibuprofen and IX 207-987; whether such effects might explain its analgesic and anti-inflammatory actions will be the subject of further studies.

Previous workers have described pharmacokinetic parameters for total car-

profen following administration of the racemic mixture to a number of species. Thus, in horses values of 250 ml $kg^{-1}$ ($Vd_{area}$), 18.1 h ($t_{\frac{1}{2}\beta}$) and 58.9 ml $min^{-1}$ (Cl) have been reported following 0.7 mg $kg^{-1}$ given intravenously (Lees *et al.*, 1994). Corresponding values in sheep are 96 and 118 ml $kg^{-1}$ ($Vd_{area}$) and 26.1 and 33.7 h ($t_{\frac{1}{2}\beta}$) after i.v. dosing with 0.7 and 4.0 m $kg^{-1}$, respectively (Welsh *et al.*, 1992). In dogs an i.v. dose of 0.7 mg $kg^{-1}$ yielded values of 180 ml $kg^{-1}$ ($Vd_{area}$), 3.83 ml $min^{-1}$ (Cl) and 8.0 h ($t_{\frac{1}{2}\beta}$) (McKellar *et al.*, 1990). In the latter study, elimination half-life was similar following oral dosing of carprofen at both 0.7 and 4.0 mg $kg^{-1}$ dose rates. In adult cows, 0.7 mg $kg^{-1}$ carprofen administered intravenously during lactation produced an elimination half-life of 30.7 h (Lohuis *et al.*, 1991) but a greater $t_{\frac{1}{2}\beta}$ (57.8 h) was reported in an earlier study following the last of five injections of racemic carprofen (Ludwig *et al.*, 1989). After a single i.v. injection the volume of distribution was 90 ml $kg^{-1}$ (both studies) and milk concentrations were very low (both studies).

For the calves used in this study, $Vd_{area}$ was somewhat greater than the values reported for adult cattle; values of 152 and 147 ml $kg^{-1}$ were obtained in 8–10 week old calves (Delatour *et al.*, 1996) and 16–17 week old animals (this study), respectively. In the present study, elimination half-life was somewhat shorter (33.8 h) than the value of 43.4 h reported for 8–10 week old calves (Delatour *et al.*, 1996). MRT was also significantly shorter in the older calves. These differences probably represent more rapid biotransformations and/or excretion of both carprofen enantiomers.

The present data demonstrate that cattle differ from horses, dogs and cats in the pharmacokinetic disposition of each carprofen enantiomer. The R:S AUC ratio in this study was 58:42. In the horse the AUC ratio, R:S, was 82:18 after i.v. dosing, in the dog after oral administration a ratio of 64:36 was obtained whilst in the cat the ratio was 69:31 and 70:30 after i.v. doses of 0.7 and 4.0 mg $kg^{-1}$, respectively (Lees *et al.*, 1991; McKellar *et al.*, 1994; Taylor *et al.*, 1995). Pharmacokinetic differences were thus qualitatively similar, with the R(−) enantiomer predominating, but quantitatively different in these four species. Further studies have shown that the enantioselective pharmacokinetics of carprofen in horses and dogs is due to inherent differences in elimination pathways and not to chiral inversion (McKellar *et al.*, 1994; P. Delatour & P. Lees, unpublished results). Moreover, several previous studies in laboratory animals and man, with a range of arylpropionic acid NSAIDs, have shown that when chiral inversion does occur, it is almost invariably from R(−) to S(+) (Caldwell *et al.*, 1988; Evans, 1992). It is thus unlikely that the differences in carprofen enantiomer disposition in calves are attributable to chiral inversion. Furthermore, studies by Graser *et al.* (1991) have shown, following *in vitro* incubation of carprofen enantiomers in the presence of cow liver homogenate supernatants, that there is no chiral inversion in either direction.

Processes governing the distribution of carprofen enantiomers into transudate and exudate do not appear to be enantioselective. Thus, R:S concentrations expressed as percentages of total concentration in exudate, transudate and plasma were, at all sampling times, very similar. Penetration into transudate was very limited and this is predictable from the low volumes of distribution of both enantiomers and the fact that they are probably highly bound to plasma protein. More rapid and greater penetration of both enantiomers into inflammatory exudate was

demonstrated in this study and this is probably explicable in terms of high binding to plasma protein (and increased blood flow to sites of acute inflammation), since exudation of plasma with its attendant proteins will be accompanied by protein-bound drug. As previously described for phenylbutazone (Higgins *et al.*, 1984a; Lees *et al.*, 1986) and flunixin (Lees & Higgins, 1984) in models of equine acute inflammation, the carprofen data indicate that the time course of drug action and the prediction of dosage schedules are more likely to be dependent on drug distribution into and persistence in inflammatory exudate than to drug plasma concentration.

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