Anti-inflammatory effects of carprofen, carprofen enantiomers, and N\textsuperscript{6}-nitro-L-arginine methyl ester in sheep

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Objective—To assess anti-inflammatory effects of carprofen (CPF), CPF enantiomers, and N\textsuperscript{6}-nitro-L-arginine methyl ester (L-NAME) in sheep.

Animals—8 sheep.

Procedure—Sheep with SC tissue cages were used. After intracaveal injection of 1% carrageenan, sheep were given single doses of racemic (Rac; 50:50 mixture of S\textsuperscript{(+)} and R\textsuperscript{(-)} enantiomers)-CPF (4.0 mg/kg), R\textsuperscript{(-)}CPF (2.0 mg/kg), S\textsuperscript{(+)}CPF (2.0 mg/kg), L-NAME (25 mg/kg), and placebo (PLB) IV in a crossover design.

Results—Rac-CPF and S\textsuperscript{(+)}CPF inhibited serum thromboxane\textsubscript{2} (TXB\textsubscript{2}) and exudate prostaglandin (PG)\textsubscript{2} generation significantly for 32 hours. Maximal inhibitory effect for serum TXB\textsubscript{2} was 79 ± 3% for Rac-CPF and 68 ± 6% for S\textsuperscript{(+)}CPF. The Rac-CPF and S\textsuperscript{(+)}CPF induced 50 to 98% reversible inhibitory effect for exudate PGE\textsubscript{2} generation during a 4- to 32-hour period. The R\textsuperscript{(-)}CPF and L-NAME attenuated serum TXB\textsubscript{2} generation significantly. The R\textsuperscript{(-)}CPF did not affect exudate PGE\textsubscript{2} production, whereas L-NAME potentiated exudate PGE\textsubscript{2} generation by 30% during 4 to 32 hours. The S\textsuperscript{(+)}CPF and L-NAME increased leukotriene B\textsubscript{4} generation and WBC recruitment in exudate although significance was achieved only at a few time points. Increase in skin temperature over inflammatory cages was effectively inhibited by Rac-CPF and S\textsuperscript{(+)}CPF but not by R\textsuperscript{(-)}CPF.

Conclusions and Clinical Relevance—Carprofen is a potent cyclooxygenase inhibitor in vivo in sheep, and its anti-inflammatory effects are attributable only to S\textsuperscript{(+)}CPF in Rac-CPF. Nitric oxide may enhance eicosanoid production and accelerate the acute inflammatory process. (Am J Vet Res 2002;63: 782–788)

Carprofen (CPF) is a non-steroidal anti-inflammatory drug (NSAID) classified as an aryl-propanoic acid. In animal models of carrageenan-induced edema, ultraviolet-induced erythema, bradykinin, and histamine-induced capillary permeability, CPF possesses greater anti-inflammatory potency than phenylbutazone, mfenamic acid, and acetyl-salicylic acid.\textsuperscript{1} It significantly decreased carrageenan-induced oedema in horses,\textsuperscript{2} whereas in cows with endotoxin-induced mastitis, Rac-CPF significantly reduced heart rate, rectal temperature, and mammary gland swelling.\textsuperscript{3} Substantial analgesic properties have also been reported for CPF in dogs\textsuperscript{4} and sheep.\textsuperscript{5} The mode of action of CPF is not fully understood, although it has been classified as a weak inhibitor of cyclooxygenase (COX).\textsuperscript{6} In rats, CPF does not substantially modify leukotriene (LT) generation, indicating that it is not an inhibitor of lipooxygenase (LOX).\textsuperscript{7} Previous studies using a SC tissue cage model of acute inflammation in dogs\textsuperscript{8} and calves\textsuperscript{9} revealed that CPF does not inhibit exudate PGE\textsubscript{2}, serum TXB\textsubscript{2}, or 12-hydroxyeicosatetraenoic acid (HETE) and indicated that CPF does not inhibit COX isoenzymes and 12-LOX in these species.

Carprofen is a chiral compound that contains an asymmetrical carbon; thus there are 2 enantiomeric forms, the S\textsuperscript{(+)} and R\textsuperscript{(-)} enantiomers. Carprofen preparations presently available for clinical use are racemic (rac) mixtures (50:50). The S\textsuperscript{(+)}CPF enantiomer is consistently a more active anti-inflammatory drug and is more toxic than the R\textsuperscript{(-)} enantiomer in rats.\textsuperscript{10} The body is a chiral environment and stereoselectivity exists in a wide range of biochemical and physiologic processes. A role for nitric oxide (NO) has been detected in acute inflammation.\textsuperscript{11} In most instances, inhibition of NO synthase (NOS) decreases inflammatory responses.\textsuperscript{12} In mice, NO is associated with inflammatory pain and inhibition of NO by nitro-L-arginine methyl ester (L-NAME) induces antinociceptive activity.\textsuperscript{13} In rats, NO is implicated in the acute inflammatory response following footpad injection of carrageenan\textsuperscript{14} or topical application of mustard.\textsuperscript{15} It is also reported that NO is directly or indirectly linked to immune complex-induced tissue injury.\textsuperscript{16} Nitric oxide synthase can be inhibited by a group of guanidino-monosubstituted derivatives of l-arginine, including l-N'–monomethyl-arginine (NMMA), L-NAME, and l-N'-amino arginine, and these drugs have become useful tools in the investigation of the pathophysiologic importance of NO.\textsuperscript{17} There is evidence that NO interacts with COX. Nitric oxide enhances the generation of prostaglandins (PG) and the COX isoenzymes are potential receptor targets for NO in a renal inflammatory model.\textsuperscript{18} In acute pancreatitis, inhibition of NOS causes an inhibition...
of 6-keto-PGF\textsubscript{1α} and thromboxane\textsubscript{A2} (TXB\textsubscript{2}) generation, and platelet eicosanoid generation is mediated through a NO-dependent mechanism.\textsuperscript{16} The purpose of the study reported here was to investigate the effects of rac-CPF, its enantiomers, and L-NAME on COX and 5-LOX derived eicosanoids, inflammatory cells, and increase in skin temperature after IV administrations of rac-CPF, R(-)CPF, S(+)-CPF, and L-NAME in vivo in sheep by use of a SC tissue cage model of inflammation.

**Materials and Methods**

Sheep—Eight male sheep weighing 63 ± 3 kg that were approximately 1.5 years old at the time the first crossover were used. Hay and water were provided ad libitum.

Experimental protocol—The experiments were carried out with a SC tissue cage acute inflammatory model, as described,\textsuperscript{20} that was adapted for sheep. Briefly, 2 months before the first cross-over experiment, 2 tissue cages were implanted SC in each side of the neck of each sheep by use of general anaesthesia (propofol and halothane). The cages were hollow spheres made from polypropylene with an external diameter of 2.0 cm and each had 6 holes (0.5 cm in diameter) cut into the surface. Acute inflammation was induced by injection of sterile carrageenan (0.3 ml, 1%) into 1 cage 15 minutes before drug administration. Inflammation was maintained by injection of 0.2 ml of sterile carrageenan (1%) into the cages 8 hours after drug or placebo administration. A cage in the other side of the neck was used for the collection of tissue transudate (extracellular fluids). A different cage was used for carrageenan injection on each of the first 4 cross-over occasions; for the fifth crossover, the cage used on the first occasion was reused.

The study was carried out as a 5-way cross-over Latin square design such that 15 minutes following injection of carrageenan into the tissue cages (time zero), each sheep received IV administration of rac-CPF (4.0 mg/kg), R(-)CPF (2.0 mg/kg), S(+)-CPF (2.0 mg/kg), L-NAME (25 mg/kg), or placebo (PLB) (saline [0.9% NaCl] solution) as a single rapid bolus via the right jugular vein, according to the cross-over design. A 4-week washout period was allowed between each crossover.

Three milliliters of venous blood for determination of serum TXB\textsubscript{2} concentration was collected into a plastic syringe 20 minutes before drug administration (–20 min) and 1, 2, 4, 6, 8, 12, 24, 32, 48, 72, 96, 120, and 144 hours after drug injection, transferred into a glass tube, and incubated in a water bath at 37 C for 90 minutes to allow clotting. The tube was centrifuged at 1,800 \( \times \) g and 4 C for 10 minutes. The supernatant (serum) was transferred into 2-ml plastic tubes and stored at –70 C until analysis of TXB\textsubscript{2} concentration. Another 1 ml of venous blood was drawn into a 1.5-ml plastic tube containing potassium EDTA\textsuperscript{21} at –20 minutes and 4, 12, 24, and 48 hours for hematologic determinations.

Exudate samples (approx 1 ml) were collected into plastic syringes by use of needle puncture from the tissue cages at ~15 minutes (transudate only) and 2, 4, 6, 8, 12, 24, 32, 48, 72, 96, 120, and 144 hours. Samples were immediately transferred into glass tubes containing 50 units of heparin and 10 µg of BW540C, a dual COX and LOX inhibitor,\textsuperscript{21} to prevent generation of eicosanoids ex vivo. The samples were centrifuged at 1,800 \( \times \) g and 4 C for 20 minutes and the supernatant decanted into 1.5-ml Eppendorf vials and stored at –70 C until analyses were performed.

Skin temperature over the tissue cages was recorded at ~20 minutes and 1, 2, 4, 6, 8, 12, 24, 32, 48, 72, 96, 120, and 144 hours by use of a direct-reading infrared thermometer\textsuperscript{22} held approximately 1 cm above each tissue cage.

**Analyses of TXB\textsubscript{2}, PGE\textsubscript{2} and leukotriene (LT)B\textsubscript{4}—** Serum TXB\textsubscript{2} and exudate PGE\textsubscript{2} concentrations were determined by use of validated radioimmunoassay (RIA) methods.\textsuperscript{23} Leukotriene B\textsubscript{4} concentrations in exudate were measured by use of a commercial RIA kit.\textsuperscript{24}

**Analyses of NO generation in exudates—**Nitrate in tissue cage fluid was converted to nitrite by the enzyme reductase and nitrite was quantified in a chemiluminescence NO analyser\textsuperscript{25} as reported.\textsuperscript{26}

**Hematologic determinations—**Hematologic variables determined in blood samples comprised numbers of WBC, erythrocytes, and platelets, and in exudate samples WBC numbers were determined. All cell counts were measured by use of an automated hematology analyzer.\textsuperscript{27}

**Pharmacodynamic modeling—**Pharmacodynamic analysis was carried out by use of a commercial software package and the data of effect versus time were fitted to a sigmoid inhibitory effect model according to the equation:

\[
E = E_{\text{max}} \times \left( \frac{T}{T + T_{50}} \right)
\]

where \( E \) is percentage inhibition of TXB\textsubscript{2}, \( T \) is time (h), \( E_{\text{max}} \) is the maximal inhibitory effect, \( T_{50} \) is time at which \( E \) declines by 50% of \( E_{\text{max}} \), and \( T \) is Hill constant.

**Drugs and reagents—**The rac-CPF injectable solution was obtained at a concentration of 50 mg/ml (wt/vol). Individual R(-) and S(+)-enantiomers were prepared by use of stereospecific crystallization\textsuperscript{28} using R(+)-α-methylbenzylamine as the chiral inducer. After 4 successive crystallizations the enantiomeric purity was 99% for R(-)-CPF and 97% for S(+)-CPF. The R(-) or S(+)-enantiomer was dissolved in the solution comprising excipient of the commercial rac-CPF at 25 mg/ml (wt/vol). The L-NAME (200 mg/ml) was prepared in sterile distilled water within 24 hours of injection in each crossover occasion and stored at 4 C until use. The BW540C was obtained from a commercial laboratory.\textsuperscript{29} Standards and antisera of TXB\textsubscript{2}, PGE\textsubscript{2} and LT\textsubscript{B}\textsubscript{4} were purchased from 1 source, and ‘H-TXB\textsubscript{2}, ‘H-PGE\textsubscript{2} and ‘H-LT\textsubscript{B}\textsubscript{4} were purchased from another source.\textsuperscript{30}

**Statistical analyses—**Results were expressed as mean ± SEM values. Analysis of variance was performed by use of the model: \( Y = \mu + \text{treatment} + \text{time} + \text{sheep} + \text{sequence} + \text{period} + \text{treatment} \times \text{time} + \text{treatment} \times \text{sheep} + \text{time} \times \text{sheep} + e \), where \( Y \) is the response variable, \( \mu \) is the overall mean, and \( e \) is the residual error. This was performed with a general linear model (GLM) program in a software package.\textsuperscript{31} The effects of ambient temperature and pretreatment values on the experimental variables were estimated via ANOVA in the GLM model. When significant differences were observed, differences of the measurements collected at each time point between treatment groups, and between pretreatment and posttreatment were identified by use of Fisher multiple comparisons. For all comparisons, a value of \( P < 0.05 \) was considered significant.

**Results**

**Assay methods—**The limits of quantification for the RIA of TXB\textsubscript{2}, PGE\textsubscript{2}, and LT\textsubscript{B}\textsubscript{4} were 5 pg/tube. The linearity of the standard curves was > 95%. The coefficients of variation for the inter- and intra-assays were < 10% for all of the RIA. The cross reactivities of specific antisera for other eicosanoids were < 5% except for anti-PGE\textsubscript{2} antiserum, which did not discriminate PGE\textsubscript{1}.

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Effects of drug- or PLB treatment on skin temperature—In the PLB-treated group, mean value for skin temperature increased by 0.43 ± 0.09 °C during a 144-hour period, and a maximal increase of 1.33 ± 0.49 °C was recorded at 12 hours. Most of the temperatures recorded in the PLB-treated group were higher than the values recorded 20 minutes before carrageenan injection; however, significance (P < 0.05) was only detected at 12 hours, compared with the preinjection value, because of large interanimal variation. Compared with the preinjection values, skin temperature increased significantly at 12 hours after R(–)CPF injection, but did not change after rac-CPF, S(+)-CPF, or L-NAME injection.

Effects of PLB or drugs on serum TXB2 generation—There were significant (P < 0.001) differences among the overall means of the 5 treatment groups for serum TXB2 generation. The treatment X time interactions were significantly (P < 0.001) different, indicating that the measurements at some time points differed significantly among treatment groups. Fisher multiple comparisons were carried out to confirm the differences. Compared with preinjection values (27.13 ± 1.86 ng/ml), PLB treatment did not alter serum TXB2 concentration at any time point, rac-CPF and S(+)-CPF treatment decreased serum TXB2 concentrations significantly between 1 and 32 hours, and R(–)-CPF inhibited serum TXB2 generation only at 6 and 8 hours. The L-NAME treatment induced small but significant inhibitory effects on serum TXB2 generation for 48 hours (Fig 1).

Inter-group comparison revealed that there were no significant differences in preinjection values among the 5 treatment groups. Following treatments, concentrations of TXB2 in the rac-CPF and S(+)-CPF groups were significantly lower than the values in the R(–)-CPF and PLB treated groups for up to 32 hours, whereas rac-CPF administration induced greater inhibitory effects than S(+)-CPF treatment did for the first 6 hours. There were no significant differences in serum TXB2 concentrations between the R(–)-CPF and PLB-treated groups. Serum TXB2 concentrations in the L-NAME-treated group were slightly lower than in the PLB-treated group, but the difference was significant only at 8 hours. The pharmacodynamic parameters for serum TXB2 inhibition were determined. (Table 1).

Effects of placebo or drugs on PGE2 generation in exudates—Before carrageenan injection into the cages, PGE2 was unquantifiable (< 0.05 ng/ml) in the tissue-cage fluid (transudate). Intracaval carrageenan injection induced PGE2 generation in the inflammatory exudate in a time-related fashion (Fig 2). The increase in PGE2 generation in the exudate was significant from 4 to 32 hours, and a peak concentration of 86.23 ± 11.75 ng/ml was achieved after 12 hours (ie, 4 hours after the second intracaval injection of carrageenan). Analysis of variance revealed that the differences in PGE2 concentrations among treatment groups were significant (P < 0.001). Further comparisons revealed that the inhibition in rac-CPF- and S(+)-CPF-treated groups was significantly (P < 0.001) different from inhibition in the PLB, R(–)-CPF, and L-NAME treated groups, that there were no significant differences between the Rac-CPF and S(+)-CPF groups or between the PLB and R(–)-CPF groups, and that PGE2 in the L-

![Figure 1—Percentage inhibition (mean ± SEM) of serum thromboxane (TXB2) after IV administration of racemic carprofen (Rac-CPF; □, 4.0 mg/kg), R(–)-CPF (▼, 2.0 mg/kg), S(+)-CPF (▲, 2.0 mg/kg), nitro-L-arginine methyl ester (L-NAME; ●, 25 mg/kg), or placebo (PLB; ◆, in 8 sheep) in which inflammation was induced by injection of carrageenan in a SC tissue cage. At each time point, values with different letters are significantly (P < 0.05) different.](image)

Table 1—Pharmacodynamic parameters (mean ± SEM) of thromboxane (TXB2) inhibition after IV administration of placebo (PLB), racemic carprofen (Rac-CPF, 4.0 mg/kg), R(–)-CPF (2.0 mg/kg), S(+)-CPF (2.0 mg/kg), or nitro-L-arginine methyl ester (L-NAME; 25 mg/kg) in 8 sheep in which inflammation was induced by injection of carrageenan in a SC tissue cage.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Rac-CPF</th>
<th>S(+)-CPF</th>
<th>R(–)-CPF</th>
<th>L-NAME</th>
<th>PLB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Emax (%)</td>
<td>78.84 ± 3.19</td>
<td>73.27 ± 6.82</td>
<td>26.32 ± 6.81</td>
<td>8.71 ± 4.74</td>
<td></td>
</tr>
<tr>
<td>IT50 (h)</td>
<td>19.00 ± 3.20</td>
<td>24.40 ± 4.89</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>γ</td>
<td>2.49 ± 0.81</td>
<td>4.77 ± 2.08</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>AUC0-last</td>
<td>1603.50 ± 135.98</td>
<td>1723.70 ± 334.93</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>

The data of percentage serum TXB2 inhibition against time were fitted to a sigmoidal inhibitory effect model. Emax = Maximal inhibitory effect. IT50 = Time at which the inhibitory effect declines by 50% of Emax. N/A = Not applicable. γ = Hill constant. AUC = Area under the curve using percentage TXB2 inhibition against time plot.
NAME-treated group was significantly ($P < 0.01$) greater than those in all other groups.

Intergroup comparisons for the values at each time point indicated that the values at 2 hours were not different among groups, PGE$_2$ concentrations in exudates were not significantly different between Rac-CPF-treated and S(+)CPF-treated groups or between PLB-treated and R(−)CPF-treated groups at any time point up to 144 hours, and the concentrations of PGE$_2$ in Rac-CPF-treated and S(+)CPF-treated groups were significantly lower than the values in the PLB-treated and R(−)CPF-treated groups from 4 to 32 hours. Compared with the PLB group, administration of L-NAME significantly increased PGE$_2$ generation in exudates at 12, 24, and 32 hours.

**Effects of placebo or drugs on LTB$_4$ generation in exudates**—Leukotriene B$_4$ was undetectable (<0.05 ng/ml) in the transudate before injection of carrageenan into the cages. Following carrageenan injection, LTB$_4$ production increased steadily in a time-related fashion with a peak concentration of 1.83 ± 0.44 ng/ml at 12 hours. There were significant ($P < 0.001$) differences among treatment groups; however, further testing by use of Fisher multiple comparisons indicated that only the value for the S(+)CPF group differed significantly from the other 4 groups ($P < 0.01$) and the values for L-NAME were significantly ($P < 0.05$) different from the values in R(−)CPF, S(+)CPF and PLB groups. Compared with the PLB group, LTB$_4$ concentrations in the S(+)CPF group were higher at most time points, but significance was achieved only at 12, 48, and 72 hours. The LTB$_4$ concentrations in the S(+)CPF group were also significantly higher than all other groups at 48 and 72 hours. The concentrations of LTB$_4$ were not significantly different among PLB, Rac-CPF, R(−)CPF, and L-NAME groups at any time point. There were no significant differences in LTB$_4$ generation among experimental sequences or periods, but there was significant ($P < 0.001$) animal variation in LTB$_4$ generation, although this did not alter the effects of the treatments (Table 2).

**Effects of placebo or drugs at inflammatory sites**—Carrageenan caused a time-related recruitment of WBC in the inflammatory exudate (Fig 3). The WBC number increased slowly after the first injection of carrageenan (5.35 ± 10$^9$ cells/L at 4 hours and 5.49 ± 1.32 × 10$^9$ cells/L at 8 hours) and steeply after the second injection. A plateau was reached in 12 to 24 hours (17.63 ± 3.72 to 17.03 ± 4.44 × 10$^6$ cells/L) before a rapid decrease occurred at 48 hours (5.33 ± 0.80 × 10$^6$ cells/L).

**Table 2**—Mean ± SEM concentrations (ng/ml) of exudate LTB$_4$ after IV administration of PLB, Rac-CPF (4.0 mg/kg), R(−)CPF (2.0 mg/kg), S(+)CPF (2.0 mg/kg) or L-NAME (25 mg/kg) in 8 sheep in which inflammation was induced by injection of carrageenan in a SC tissue cage.

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>PLB</th>
<th>Rac-CPF</th>
<th>R(−)CPF</th>
<th>S(+)CPF</th>
<th>L-NAME</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre</td>
<td>U/D</td>
<td>U/D</td>
<td>U/D</td>
<td>U/D</td>
<td>U/D</td>
</tr>
<tr>
<td>2</td>
<td>0.41 ± 0.09</td>
<td>0.78 ± 0.15</td>
<td>0.52 ± 0.08</td>
<td>0.94 ± 0.19</td>
<td>1.00 ± 0.23</td>
</tr>
<tr>
<td>4</td>
<td>0.85 ± 0.10</td>
<td>0.89 ± 0.11*</td>
<td>0.84 ± 0.19</td>
<td>1.14 ± 0.19*</td>
<td>1.48 ± 0.17*</td>
</tr>
<tr>
<td>8</td>
<td>1.28 ± 0.19*</td>
<td>1.05 ± 0.05*</td>
<td>1.00 ± 0.23*</td>
<td>1.74 ± 0.31*</td>
<td>1.19 ± 0.21*</td>
</tr>
<tr>
<td>12</td>
<td>1.35 ± 0.44*</td>
<td>1.88 ± 0.34*</td>
<td>1.35 ± 0.31*</td>
<td>2.85 ± 0.87*</td>
<td>2.57 ± 0.92*</td>
</tr>
<tr>
<td>24</td>
<td>1.06 ± 0.15*</td>
<td>1.62 ± 0.18*</td>
<td>0.97 ± 0.18*</td>
<td>1.31 ± 0.17*</td>
<td>1.58 ± 0.43*</td>
</tr>
<tr>
<td>32</td>
<td>1.21 ± 0.15*</td>
<td>1.42 ± 0.22*</td>
<td>1.24 ± 0.23*</td>
<td>1.56 ± 0.30*</td>
<td>1.42 ± 0.17*</td>
</tr>
<tr>
<td>48</td>
<td>1.33 ± 0.19*</td>
<td>1.26 ± 0.15*</td>
<td>1.12 ± 0.25*</td>
<td>2.71 ± 1.02*</td>
<td>1.49 ± 0.29*</td>
</tr>
<tr>
<td>72</td>
<td>1.07 ± 0.24*</td>
<td>1.25 ± 0.11*</td>
<td>0.89 ± 0.15*</td>
<td>2.70 ± 0.76*</td>
<td>1.42 ± 0.27*</td>
</tr>
<tr>
<td>96</td>
<td>0.90 ± 0.24*</td>
<td>1.06 ± 0.15*</td>
<td>1.14 ± 0.22*</td>
<td>1.59 ± 0.29*</td>
<td>1.76 ± 0.61*</td>
</tr>
<tr>
<td>120</td>
<td>0.96 ± 0.15*</td>
<td>1.03 ± 0.19*</td>
<td>1.48 ± 0.68*</td>
<td>1.39 ± 0.51*</td>
<td>1.12 ± 0.27*</td>
</tr>
<tr>
<td>144</td>
<td>0.83 ± 0.09</td>
<td>1.26 ± 0.15*</td>
<td>1.21 ± 0.25*</td>
<td>1.03 ± 0.26*</td>
<td>1.23 ± 0.23*</td>
</tr>
</tbody>
</table>

Pre = Before administration of carrageenan. U/D = Undetectable. *Significant ($P < 0.05$) difference compared with values determined prior to carrageenan injection. †Significant ($P < 0.05$) difference, compared with sheep treated with PLB, CPF, or R(−)CPF.
There were significant differences among treatment groups; however, further intergroup comparisons revealed that there was no significant difference in WBC accumulation among the PLB, Rac-CPF, R(−)CPF, and S(+)CPF treatment groups, and only the L-NAME group values were significantly greater than those of the other 4 groups. Compared with the PLB-treated group, IV injection of Rac-CPF, R(−)CPF, S(+)CPF, and L-NAME led to a slight decrease at 12 hours that was followed by a moderate increase at 24 hours, at which time a significant difference was observed between values in the Rac-CPF- and L-NAME-treated groups (Fig 3).

Effects of placebo or drugs on WBC and platelet numbers in blood—Intravenous administration of Rac-CPF, R(−)CPF, S(+)CPF, L-NAME, and PLB did not modify the numbers of WBC and platelets in the venous blood at any time point.

Effects of L-NAME on NO production in inflammatory exudates—Nitrite was undetectable in exudate and transudate before nitrate was converted to nitrite. This indicated that most of the NO products in the tissue-cage fluids in the sheep were present in the form of nitrate (Fig 4). Before carrageenan injection, mean concentration of nitrite in the tissue-cage fluids was 2.56 ± 0.32 µM. After intracaval injection of carrageenan, mean concentrations of nitrite increased to 5.80 ± 1.02 µM at 2 hours and the concentrations were significantly (P < 0.01) increased for 48 hours compared with the preinjection values. Intravenous administration of L-NAME increased NO concentration from 2.56 ± 0.32 to 7.03 ± 0.50 µM at 2 hours, and this value was significantly higher than that of the PLB-treated group.

**Discussion**

Our study in sheep revealed that IV administration of rac-CPF and S(+)CPF inhibited serum TXB2 and exudate PGE2 generation significantly for 32 hours, and the inhibitory effects declined in a time-related fashion. The maximal inhibitory effects on these 2 COX products were > 68% of control values, which suggests that rac-CPF and S(+)CPF are effective COX inhibitors and that they induce their anti-inflammatory effects at least in part by inhibition of COX. This differs from previous studies in which CPF did not have significant inhibitory effects on carrageenan-induced PGE2 in exudate or serum TXB2 generation in dogs, calves, and horses. This may be a species-specific finding, or it may be a reflection of the administration route and dose rate. The dose rate used in the studies in horses and calves was 0.75 mg/kg for rac-CPF, which was considerably less than used in our study. The dose rates used in dogs were 4.0 mg/kg for rac-CPF and 2.0 mg/kg for R(−)CPF and S(+)CPF; however, the drugs were given orally and relatively low plasma drug concentrations were measured (Cmax was 31.2 µg/ml for rac-CPF and 13.6 µg/ml for S(+)CPF). The plasma Cmax for rac-CPF, 31.2 µg/ml, failed to inhibit serum TXB2 generation by platelet COX (COX-1) during blood clotting. In the study reported here, plasma concentrations of CPF were measured in 5 sheep by use of derivatization and high-performance liquid chromatography as described and results indi-
icated that the plasma concentration 5 minutes after IV administration of rac-CPF (4.0 mg/kg) was 39.9 µg/ml for R(-)CPF and 33.3 µg/ml for S(+)-CPF. The IC₅₀ value for COX in ovine seminal vesicles (COX-1) is 1 mg/ml (48 µM) for rac-CPF, which is less than which is less than the concentrations detected in our study. Clearly a species difference exists between dogs and sheep, although differences in protein binding of the drug between in vitro and in vivo studies may affect the results. Carprofen and S(+)-CPF have been described as weak inhibitors of COX in several standard rodent models of inflammation. The IC₅₀ of rac-CPF for the COX from ovine seminal vesicles is 48 µM, whereas the drug concentration that induces 50% of Eₘₐₓ (IC₅₀) of indomethacin is 0.5 µM, and CPF is also approximately 100 times less potent at inhibiting COX activity in inflammatory cells (human synovial cells and rat peritoneal polymorphonuclear cells) than indomethacin. Aryl-propionic NSAID, including CPF, contain an asymmetric carbon atom and therefore exist as 2 enantiomers. There are often substantial differences in potency and biological effects between enantiomeric pairs in vivo. Generally, S(+) enantiomers are more potent than R(-) enantiomers for inducing effects, as has been detected for CPF in rats and mice and ketoprofen in several experimental animal species, humans, and calves. The anti-inflammatory differences between the enantiomeric pairs of rac-CPF have not been detected in domestic animal species because rac-CPF and its enantiomers did not have any significant effects on the inflammatory mediators that have been studied. Results of our study confirm that the anti-inflammatory effects of the enantiomers of CPF were different, and that S(+)CPF was the eutomer and R(-)CPF was the disommer in sheep. The inhibitory effects of rac-CPF on serum TXB₂ generation were significant but small (approximately 8%), which indicates that R(-)CPF contributes minimally to the inhibitory effects of rac-CPF on serum TXB₂ generation. Administration of R(-)CPF had little effect on PGE₂ generation in exudate. It is, therefore, likely that after administration of rac-CPF, R(-)CPF does not contribute to therapeutic effects in inflammation and that S(+)-CPF confers the therapeutic effects of rac-CPF treatment.

Previous studies revealed that CPF is an inhibitor of phospholipase (PLA₂), and is more potent than the other NSAID as an inhibitor of arachidonic acid release from cellular lipids. Our study failed to support this finding. Administration of rac-CPF and its active enantiomer, S(+)CPF, led to slightly increased LTB₄ concentrations in exudate, thus indicating that inhibition of PLA₂ did not occur, but that suppression of the COX enzyme may have led to a slight shift of arachidonic acid metabolism.

Nitric oxide is involved in mammalian immune defence mechanisms and has a role in inflammation. Inhibition of NO synthase by guanidino-monosubstituted derivatives of L-arginine, including L-NNAME and L-NAME, leads to decreased inflammatory responses. In our study in sheep, IV administration of L-NNAME caused an increase in NO formation in exudate. This suggests surprising species differences. The mechanism underlying this finding is unclear. Previous reports indicate that L-NNAME, which is a potent inhibitor of endothelial NO synthesis, does not affect NO production in neutrophils. In our acute inflammation model, a large portion (59.69 ± 4.63%) of the inflammatory cells that accumulated in the exudate were neutrophils; although this could explain a low response, it cannot explain the short but significant increase in NO generation after administration of L-NNAME. It is known that L-NNAME is a nonspecific inhibitor of the 3 isoforms of NOS (neuronal, endothelial, and inducible) and it reverses bradykinin-induced endothelial relaxation in sheep digital vessels in vitro. However, it is possible that L-NNAME was rapidly converted into L-arginine by the metabolic enzyme systems in our sheep, providing more substrate for biosynthesis of NO. A metabolic study is required to address this issue. In inflammatory exudate, NO products (PGE₂ and LTB₄) increased simultaneously. This suggests that COX and LOX may respond to NO and the pro-inflammatory effects of NO may be amplified by increased PGE₂ and LTB₄.

References


