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Studies on Pharmacokinetics and Pharmacodynamics of Phenylbutazone, Flunixin Meglumine, Carprofen and Paracetamol in Some Domesticated Animal Species

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A thesis submitted for the degree of doctor of philosophy

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Contents

Contents I

Abbreviations III

Pharmacokinetic and pharmacodynamic parameters VI

Pharmacokinetic and pharmacodynamic terms IX

Acknowledgements XI

Declaration XII

Summary XIII

Chapter 1 1
General introduction

Chapter 2 32
General materials and methods.

Chapter 3 53
Pharmacokinetic and pharmacodynamic studies of flunixin meglumine and phenylbutazone in sheep.

Chapter 4 89
Pharmacological effects of carprofen and its enantiomers in sheep.

Chapter 5 112
The roles of nitric oxide and its interactions with eicosanoid generation in acute inflammation in sheep.

Chapter 6 129
Pharmacokinetics and pharmacodynamics of phenylbutazone and flunixin meglumine in donkeys.

Chapter 7 148
Pharmacokinetic and pharmacodynamic studies on phenylbutazone and oxyphenbutazone in goats.

Chapter 8 159
Comparative pharmacokinetics of paracetamol (acetaminophen) and its sulphate and glucuronide metabolites in desert camels and goats.

Chapter 9 174
General discussion.

References 188

Appendix

Appendix A 215

Appendix B 230

Appendix C 245

Appendix D 249

Appendix E 254

Abbreviations

AIC	Akaike information criterion
ANOVA	analysis of variance
°C	degrees centigrade
Co-Var	coefficient of variation
COX	cyclooxygenase
CPF	carprofen
$cPLA_2$	cytoplasmic phospholipase A_2
cNOS	constitutive form of nitric oxide synthase
cpm	counts per minute
et al.	and others
Fig	figure
FM	flunixin meglumine
g	gramme
g	gravity, $10^{-11}N.m/s^2$
GLM	general linear model
h	hour
HETE	hydroxyeicosatetraenoic acid
HPETE	hydroperoxyeicosatetraenoic acid
HPLC	high performance liquid chromatography
5-HT	5-hydroxytryptamine
IL	interleukin
i.m.	intramuscular or intramuscularly
iNOS	inducible form of nitric oxide synthase
i.v.	intravenous or intravenously
kg	kilogramme

KPF	ketoprofen
l	litre
L-NAME	N^G-nitro-L-arginine methyl ester
log10	logarithm based on 10
LOX	lipoxygenase
LPS	lipopolysaccharides
LT	leukotriene
M	mole
MAICE	minimum Akaike information criterion estimation
mg	milligramme
min	minute
ml	millilitre
ng	nanogramme
NMMA	N^G monomethyl-arginine
NO	nitric oxide
NOS	nitric oxide synthase
NSAID	nonsteroidal antiinflammatory drug
OPBZ	oxyphenbutazone
PAF	platelet activating factor
PBS	phosphate buffered saline
PBZ	phenylbutazone
PD	pharmacodynamic or pharmacodynamics
PG	prostaglandin
PGG	prostaglandin G
PGH	prostaglandin H
PGI_2	prostacyclin
pH	negative logarithm of hydrogen ion concentration

PK	pharmacokinetic or pharmacokinetics
PL	phospholipase
PLB	placebo
PLT	platelets
PMN	polymorphonuclears
p.o.	oral or orally
PPB	minus background
PRT	paracetamol
r	correlation coefficient
R^2	square of correlation coefficient.
Rac	racemic
RIA	radioimmunoassay
SD	standard deviation
SE	standard error of the mean
$sPLA_2$	secretory phospholipase A_2
SSV	sheep seminal vesicles
TNF	tumour necrosis factor
TX	thromboxane
μg	microgram
μM	micromole
WBC	white blood cells

Pharmacokinetic and pharmacodynamic parameters

A, P, B	The zero time intercepts associated with the α, π, and β phase, respectively, following intravenous administration.
α, π, β	"Macro" rate constants associated with the distribution, slow distribution phase and elimination phase, respectively, following intravenous administration.
AUC	Area under the curve.
$AUC_{0\text{-last}}$	AUC computed to the last observation.
$AUC_{0\text{-}\infty}$	AUC extrapolated to infinity
AUMC	Area under the moment curve.
$AUMC_{0\text{-last}}$	AUMC computed to the last observation.
$AUMC_{0\text{-}\infty}$	AUMC when the time concentration curve is extrapolated to infinity.
Cl_B	Total body clearance. Cl_B= Dose/AUC.
C_{max}	The peak or maximum drug concentration in plasma following extravascular administration or the maximum concentration in tissue-cage fluids following intravenous administration.
C	Drug concentration.
C_p^0	Initial concentration of the drug in plasma following an intravenous bolus administration calculated from the sum of intercepts.
D	Dose.
E	Effect.
EC_{50}	The drug concentration which produces 50% of maximum effect at the effect site.
E_{max}	Maximum effect attributed to the drug.
F	Bioavailability calculated by dividing AUC following oral administration by AUC following intravenous administration.
$F_{0\text{-last}}$	F which is associated with $AUC_{0\text{-last}}$.
$F_{0\text{-}\infty}$	F which is associated with $AUC_{0\text{-}\infty}$.

γ	Hill constant.
IC_{50}	The drug concentration which produces 50% of maximum inhibition achieved at the effect site.
k_{01}	The rate at which the drug enters the central compartment from outside the system. The absorption rate.
k_{10}	The rate at which the drug leaves the system from the central compartment. The elimination rate.
k_{12}, k_{21}	First-order transfer rate constants calculated for drug distribution between the central and peripheral compartments of a two-compartment model.
k_{12}, k_{13}, k_{21}, k_{31}	First-order transfer rate constants calculated for drug distribution between the central and peripheral compartments of a three-compartment model.
M	Hybrid parameter of one compartmental model with first order input.
MAT	Mean absorption time for a drug from an administered site into the central compartment or mean distribution time for a drug from central (plasma) compartment into tissue-cage fluid.
$MAT_{0\text{-}last}$	MAT calculated based on $MRT_{0\text{-}las}$.
$MAT_{0\text{-}\infty}$	MAT calculated based on $MRT_{0\text{-}\infty}$.
MRT	Mean residence time. It is the average amount of time a particle remains in a compartment or system
$MRT_{0\text{-}last}$	MRT when the drug concentration profile is not extrapolated to infinity, but rather is based on values up to and including the last measured concentration. $MRT_{0\text{-}last} = AUMC_{0\text{-}last} / AUC_{0\text{-}last}$.
$MRT_{0\text{-}\infty}$	MRT when the drug concentration profile is extrapolated to infinity. $MRT_{0\text{-}\infty} = AUMC_{0\text{-}\infty} / AUC_{0\text{-}\infty}$
$t_{\frac{1}{2}\alpha}$	Distribution half life following intravenous administration calculated from 0.693/α.
$t_{\frac{1}{2}\beta}$	Elimination half life following intravenous administration calculated from 0.693/β.
$t_{\frac{1}{2}\pi}$	Distribution half life following intravenous administration calculated from 0.693/π.
$t_{\frac{1}{2}k01}$	Absorption half life following extravascular administration or distribution half life from plasma compartment into tissue-cage

fluids. It is calculated from 0.639/k01.

$t_{\frac{1}{2}k10}$	Elimination half life following extravascular administration or distribution half life from plasma compartment into tissue-cage fluids. It is calculated from 0.639/k10.
t_{max}	Time at which the C_{max} is reached following an extravascular administration or time at which the C_{max} in tissue-cage fluids is reached following an intravenous administration.
V_c	Apparent volume of central compartment.
V_{ss}	Volume of distribution at steady state. $V_{ss} = MRT \times Cl_B$

Pharmacokinetic and pharmacodynamic terms

Absorption rate	The rate at which the drug is absorbed into the central compartment.
Akaike criteria	A measure of goodness of fit based on maximum likelihood. When comparing several models for a given set of data, the model associated with the smallest value of Akaike criteria is regarded as giving the best fit out of that set of models. Akaike Criteria is only appropriate for use when comparing models using the same weighting scheme. AIC = NOBS × LOG(WSS) + 2 × NPARM where NOBS is number of observations, NPARM is number of parameters and WSS is weighted sum of squared residuals.
Compartments	Many biological systems are made up of a finite number of homogenous, well mixed subsystems called compartments. Classically compartments have been used to represent blood plasma, kidneys, lungs or other organs.
Curve stripping	A graphical method for estimating parameters associated with models that can be written as a sum of exponential. This method can be used to determine initial estimates required as a prelude to non-linear least-squares regression.
Distribution rate	The rate at which the drug is distributed between compartments.
Elimination rate	The rate at which the drug is eliminated from a compartment.
Extravascular	Drug administration via routes other than directly into the blood stream. Oral, intramuscular and topical are examples of extravascular administration.
First-order	Used to denote rate constants when the transfer rates are proportional to the amount in the source compartment.
Initial estimates	Starting values for parameters. All iterative estimation procedures require initial estimates of the parameters.
Lag time	Lag time is a time delay between drug administration and the onset of drug absorption. An example of such a process is stomach emptying time following oral administration of a drug which is not absorbed from the stomach. When a drug is administered by bolus intravenous injection there is no lag phase

Least squares fitting	In least squares fitting, the estimates are those which minimise the sum of the squares of the deviations between the observed values and the values predicted by the model.
Nelder-Mead simplex algorithm	A model fitting algorithm which does not require the estimated variance - covariance matrix as part of its algorithm.
Pharmacodynamic models	Models which are used to model effect as a function of a drug concentration
Pharmacokinetic models	Models which are used to model drug concentrations as a function of time.
PK / PD link model	A pharmacokinetic / pharmacodynamic link model uses the pharmacokinetic concentration data to estimate concentrations at an effect site which are then used to model the pharmacodynamics.
Secondary parameters	Parameters which are functions of the primary model parameters.
Sigmoid	The "S" shaped nature of certain curves.
Simulation	Predict the drug concentrations from given model and time points.

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Declaration

The studies described in this thesis were carried out in the Department of Veterinary Pharmacology at the University of Glasgow Veterinary School.

The author was responsible for all results except where it is stated otherwise.

No part of this thesis has been presented to any university but it has been reproduced in parts in the following scientific papers.

Cheng, Z., McKellar, Q., Nolan, A. & Lees, P. (1996) Pharmacokinetic and pharmacodynamic studies on flunixin meglumine in donkeys. Veterinary Research Communications, **20,** 469-472.

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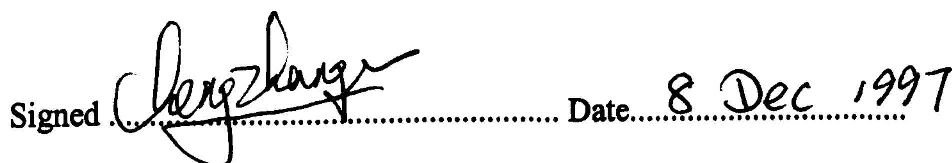
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Summary

The present study was conducted to investigate the pharmacokinetics (PK) and pharmacodynamics (PD) of some nonsteroidal antiinflammatory drugs (NSAIDs), including phenylbutazone (PBZ, in sheep, goats and donkeys), flunixin meglumine (FM, in sheep and donkeys), carprofen and its enantiomers (CPF, in sheep), paracetamol (PRT, in goats and camels) and N^G-nitro-L-arginine methyl ester (L-NAME, in sheep).

Eight sheep were prepared with subcutaneous tissue-cages and acute inflammation was induced by injection of 0.3 ml of 1 % carrageenan into the cages and maintained by a further injection of 0.2 ml carrageenan after 8 hours. Flunixin meglumine (FM, 1.1 mg/kg) and PBZ (4.4 mg/kg) were administered intravenously (i.v.) as a single dose in the sheep. Plasma kinetics showed that elimination of PBZ was slow, indicated by an elimination half life ($t_{\frac{1}{2}\beta}$) of 17.92 ± 1.74 h, a body clearance (Cl_B) of 4.56 ± 0.53 ml/kg/h and an area under concentration time curve (AUC_{last}) of 963.54 ± 95.35 µg/ml.h. The elimination of FM ($t_{\frac{1}{2}\beta}$ = 2.33 ± 0.18 h, Cl_B = 39.67 ± 4.27 ml/kg/h and AUC_{last} = 30.56 ± 3.41 µg/ml.h) was faster than PBZ. Both PBZ and FM distributed well into exudate and transudate although the penetration was slow, indicated by maximal drug concentration (C_{max}) for PBZ of 22.32 ± 1.29 µg/ml at 9.50 ± 0.73 h (exudate) and 22.07 ± 1.57 µg/ml at 11.50 ±1 .92 h (transudate), and C_{max} for FM of 1.82 ± 0.22 µg/ml at 5.50 ± 0.73 h (exudate) and 1.58 ± 0.30 µg/ml at 8.00 h (transudate), and a high mean tissue-cage fluid : plasma AUC_{last} ratio obtained in the PBZ and FM groups (80-98 %). These values are higher than previous reports in horses (Lees *et al.*, 1986) and calves (Landoni *et al.*, 1995a) using the same or higher dose rates. Elimination of PBZ and FM from exudate and transudate was slower than from plasma. Consequently the drug concentrations in plasma were initially higher and subsequently lower than in exudate and transudate. Intra-caveal injection of carrageenan induced the generation of exudate prostaglandin (PG) E_2 from an undetectable level (< 0.5 ng/ml) to 35.65 ± 4.92 ng/ml at 12 h and was accompanied by significant increases ($P<0.05$) in leukocyte numbers (41.96 ± 8.59 × 10^9 cell/L at 24 h), skin temperature over the inflamed cages (increased by 1.06 ± 0.45 °C at 12 h) and exudate leukotriene (LT) B_4 concentration (1.63 ng/ml at 12 h). Phenylbutazone produced partial inhibition of serum thromboxane (TX) B_2 and exudate PGE_2 generation and the inhibitory effect was significant for up to 32 h for serum TXB_2, and at 4 and 12 h for

exudate PGE_2. The maximal inhibitory effects (E_{max}) for serum TXB_2 was 80 ± 6 % and the IC_{50} (drug concentration which produces 50 % of E_{max}) was 10.86 ± 1.81 μg/ml and occurred at 29.96 ± 2.62 h. A maximal exudate PBZ concentration of 22.32 ± 1.29 μg/ml only produced about 10 % inhibitory effect against exudate PGE_2 generation. Phenylbutazone did not inhibit the skin temperature rise over the inflamed cages or the recruitment of exudate white blood cell (WBC) numbers significantly ($P>0.05$). Intravenous injection of FM abolished or significantly ($P<0.05$) inhibited serum TXB_2 and exudate PGE_2 formation for up to 32 h ($P<0.05$). The E_{max} for exudate PGE_2 and serum TXB_2 generation was 100 %. The IC_{50} was estimated to be 0.0053 ± 0.0032 μg/ml and 0.00019 ± 0.00012 μg/ml against serum TXB_2 and exudate PGE_2 generation, respectively. Flunixin meglumine also significantly inhibited skin temperature over the cages and accumulation of exudate WBC numbers ($P<0.05$). Phenylbutazone and FM slightly increased the exudate LTB_4 generation, indicating that these NSAIDs are not 5-lipoxygenase (5-LOX) inhibitors and that a metabolic diversion of arachidonic acid occurred. This experiment suggests that PBZ and FM have distinct effects on carrageenan-induced cycooxygenase (COX) and platelet COX. Flunixin meglumine but not PBZ is a potent COX inhibitor and antiinflammatory drug in sheep and a dose rate of 1.1 mg/ml of FM may be sufficient to produce antiinflammatory effect.

A single dose rate of racemic (rac)-CPF (4.0 mg/kg), R(-)CPF (2.0 mg/kg), S(+)CPF (2.0 mg/kg) and L-NAME (25 mg/kg) were administered i.v. to the same sheep as described above for the assessment of their antiinflammatory effects. Racemic-CPF and S(+)CPF inhibited serum TXB_2 and exudate PGE_2 generation significantly for up to 32 h ($P<0.05$). The E_{max} for serum TXB_2 was 79 ± 3 % for rac-CPF and 68 ± 6 % for S(+)CPF. Racemic CPF inhibited over 50 % of E_{max} for serum TXB_2 for up to 19.00 ± 3.20 h and S(+)CPF inhinbited over 50 % of E_{max} for up to 24.40 ± 4.89 h. Compared with placebo treated group, rac-CPF and S(+)CPF produced 50-98 % inhibition for exudate PGE_2 generation over a 4-32 h period. The inhibitory effect of rac-CPF for serum TXB_2 was significantly higher than that of S(+)CPF ($P<0.05$) in spite of small difference while the inhibitory potency for exudate PGE_2 between rac-CPF and S(+)CPF was similar ($P>0.05$). R(-)-carprofen attenuated serum TXB_2 generation by < 10 % while it potentiated exudate PGE_2 generation by 5-30 % over a period of 4-32 h. Racemic CPF and S(+)CPF enantiomer slightly increased LTB_4 generation and leukocyte recruitment in the exudate although statistic significance was achieved only at a few time points. The increase in skin temperature over inflammatory cages was effectively

inhibited by rac-CPF and S(+)CPF but not by R(-)CPF. This study indicates that CPF is a potentially useful NSAID in sheep and that the S(+) enantiomer only acts as a conventional NSAIDs for its the antiinflammatory effects. Intra-caveal injection of carrageenan induced increased nitric oxide (NO) generation by 89 ± 9 % in the exudate over a 96 h period. Administration of L-NAME upregulated the exudate nitric oxide production with a maximal increase of 21 % at 2 h ($P<0.05$). It inhibited serum TXB_2 generation by 19 ± 2 % over a 72 h period whereas it increased PGE_2 generation by 34 %, LTB_4 generation by 35 % and leukocyte recruitment by 50 % in the inflammatory exudate. This suggests that L-NAME may be converted into L-arginine in sheep in the metabolic process and that NO may play a role in acute inflammation by upregulating the activity of COX-2, 5-LOX and leukocyte recruitment.

Phenylbutazone (4.4 mg/kg) and FM (1.1 mg/kg) were given i.v. to 3 donkeys prepared with subcutaneous tissue-cages and in which carrageenan-induced acute inflammation had been developed. The elimination of PBZ from plasma was very fast, indicated by a $t_{\frac{1}{2}\beta}$ of 0.63 ± 0.13 h, a mean residence time (MRT_{last}) of 0.69 ± 0.12 h, a Cl_B of 214.18 ± 18.04 ml/kg/h and a AUC_{last} of 19.21 ± 2.11 µg/ml.h. The conversion of PBZ to oxyphenbutazone (OPBZ) was rapid and extensive (AUC ratio for OPBZ to PBZ = 67 %). The elimination of FM from the plasma in the donkeys ($t_{\frac{1}{2}\beta}$ = 2.09 ± 0.45 h and Cl_B = 50.27 ± 9.42 ml/kg/h) was similar to the time reported in the horses ($t_{\frac{1}{2}\beta}$ = 1.94 ± 0.10 h and Cl_B = 57.3 ± 2.9 ml/kg/h). The elimination of PBZ and FM in exudate was slower than in plasma. The AUC ratio for exudate to plasma for PBZ (42 %) was similar to that of FM (47 %) while the ratio for transudate to plasma for PBZ (26 %) was smaller than that for FM (55%). Phenylbutazone and FM inhibited the generation of serum TXB_2 and exudate PGE_2, and skin temperature over inflammatory foci significantly in a time-related fashion ($P<0.05$) while they did not modify leukocyte numbers and 12-hydroxyeicosatetraenoic acid (12-HETE) significantly ($P>0.05$). This study indicates that PBZ and FM are potent antiinflammatory drugs in donkeys, the dosage of PBZ should be higher and dosage interval shorter in donkeys compared to horses whereas the dose rate of FM used in horses can be extended to donkeys although tolerance studies are required to ensure safety in the donkey.

Phenylbutazone was administered i.v. and orally (p.o.) to 6 goats at a single dose rate of 4.4 mg/kg. The disposition and bioavailability (F) of PBZ and the disposition of its active

metabolite, OPBZ in plasma were investigated. The effect of PBZ administration on serum TXB_2 generation was studied. The elimination of PBZ was slow, indicated by a $t_{\frac{1}{2}\beta}$ of 15.34 ± 1.15 h and 21.99 ± 3.32 h after i.v. and p.o. administration, respectively. Following PBZ paste administration p.o., F was 61 ± 7 % (corrected by the $t_{\frac{1}{2}\beta}$) and a relatively slow absorption was observed, illustrated by a time to maximum drug concentration (tmax) of 3.47 ± 0.39 h and a mean absorption time (MAT) of 10.43 ± 8.61 h. The concentration of OPBZ in plasma was low throughout and AUC ratios of OPBZ to PBZ were approximately 2 % after i.v. and p.o. administration. Thromboxane B_2 generation in the platelets was significantly inhibited ($p<0.05$) from 1 to 12 h following i.v. and from 2 to 12 h after p.o. administration. The E_{max} was 70.63 ± 11.96 % for i.v. and 61.06 ± 11.83 % for p.o. administration.

Paracetamol was administered at dose rates of 5 mg/kg to camels and 10 mg/kg to goats by the i.v. and intramuscular (i.m.) routes. Parent paracetamol had a significantly slower clearance (21.9 ± 1.4 ml/kg/min vs 52.8 ± 7.3 ml/kg/min) ($P < 0.01$) in camels than goats. In camels the predominant metabolite in plasma was the sulphate although the ratios of glucuronide : paracetamol and sulphate : paracetamol were similar (5.20 ± 0.50 vs 6.59 ± 0.51) following i.v. administration. In goats the glucuronide metabolite was the predominant moiety in plasma and the AUC of the sulphate was only 4 % of that of the glucuronide conjugate. The apparent AUC for paracetamol in the camels following i.m. administration was larger than that following i.v. administration, however when the F was determined with correction for altered $t_{\frac{1}{2}\beta}$ within animal and between study phases it was 71 ± 17 % in goats and 105 ± 26 % in camels.

Chapter 1

General introduction

1.1 Inflammation

Inflammation may be defined as a protective response to the injury and is essentially a beneficial pathological process. It can occur in all tissues and organs in the body and the causal stimuli are diverse and include physical, chemical and biological factors. Each type of stimulus provokes a characteristic pattern of response that represents a relatively minor variation on a theme. The responses are usually accompanied by the familiar clinical signs of heat, redness, pain and swelling and were described by Celsus (30 BC—AD 38). If the stimulus persists, the fifth cardinal sign of inflammation, loss of function, may appear as tissue degeneration and fibrosis develops. Inflammation is a common pathological process involved in many diseases in animals. Examples seen daily in veterinary clinical practice include laminitis, dermatitis, enteritis, tendonitis, mastitis, metritis, pneumonitis, myositis, bursitis and countless of other ailments all characterised by the suffix—itis.

Many different mechanism are involved in the inflammatory process (Gallin *et al.*, 1992; Kelly *et al.*, 1993; Higgins & Lees, 1984d). The major responses can be classified as 1) vascular, 2) cellular, 3) formation and release of chemical mediators and 4) tissue degeneration and fibrosis. The first three processes are mainly associated with the acute phase and the fourth process is associated with the development of chronic inflammation (Fig. 1.1.).

It is now recognised that that the inflammatory mediators play a pivotal role in the inflammatory processes. Inflammatory mediators are a large family of biochemical substances which are formed and released during the inflammatory reaction and possess biological effects which initiate and maintain the inflammatory process. They include histamine, bradykinin, LTs, PGs, PAF and cytokines. Recently a number of new chemical mediators have been found and established as inflammatory mediators, including NO and families of adhesion molecules. Undoubtedly there are other mediators awaiting discovery.

1.2. Pharmacology of inflammation

1.2.1. Vascular responses

Inflammation is normally a localised protective response which serves to destroy, dilute, or wall-off both the injurious agent and the injured tissue. The early events which occur in response to an inflammatory stimulus are changes in blood flow through the affected tissues and an increase in vascular permeability. These events facilitate the exudation of plasma proteins and the immigration of leukocytes from the vascular fluid into the tissues. Alteration of blood flow also accompanies the early stage of inflammation. Following induction of inflammation, an initial constriction of the arterioles due to a vasomotor reflex which last only for a short time, is followed by a widespread dilation of venules and lymphatics. This response is mediated by vasoactive agents liberated from locally damaged tissues, which are now collectively named inflammatory mediators. Inflammatory mediators play an important role in initialising and maintaining the vascular dilation and hence increasing vascular permeability. Dilation of the blood vessels causes an increased blood flow through the affected tissues, but eventually the rate of flow decreases. It is during the time of decreased blood flow rate that the circulating leukocytes begin to adhere to the vascular endothelium, a process know as "pavementing" in preparation for migration into the extravascular tissues.

Increased vascular permeability is a result of the opening of the vascular endothelial cell junction. This is brought about by the contraction of endothelial cells, with the tough underlying basement membrane normally remaining intact. Gaps appear between endothelial cells, the cell body bulges into the lumen of vessel, the nucleus become rounded and nuclear and cell membranes crenellate (Majno & Palade, 1961). The mechanism for the development of endothelial cell constriction is rather complicated and a number of chemical mediators are involved. Vascular responses are mediated by a number of biologically active substances (Fig 1.2.). The early reports indicated that histamine from adjacent tissue mast cell is the first substance to be released. The initial increased vascular permeability and dilatation seems to be maintained at first by kinins, 5-HT and the complement system and sequentially by products deriving from arachidonic acid under the action of a fatty acid COX enzyme. The demonstration

that two soluble mediators (bradykinin and PGs) are required to influence blood flow and vascular permeability and that the presence of only one will not produce the symptoms of inflammation (Williams, 1979) highlights the ability of the body to regulate its own response to stimuli.

Increase in vascular permeability permits exudation of plasma proteins and inflammatory cells into the extravascular tissues. The accumulation of the fluids and cells at the damaged site is now collectively termed the inflammatory exudate. Unlike transudate or oedema fluids, this inflammatory exudate contains large amount of proteins, inflammatory cells and inflammatory mediators.

1.2.2. Cellular response

The accumulation and subsequent activation of leukocytes in the affected sites is an important event in the pathogenesis of virtually all forms of inflammation. In order to minimise or remove the injury from the body, it must serve to provide, as soon as possible, killers (including phagocytes and antibodies) to the site of injury. Infiltration of neutrophils and PLT occurs first. Polymorphonuclear leukocytes predominate in the early stages of acute inflammation and are the characteristic cell of the acute inflammatory process but their predominance is short-lived since they can not reproduce. Subsequently mononuclear phagocytes invade and predominate. These cells are capable of replication at the site of inflammation (Spector & Ryan, 1970). During resolution or progression to chronicity, macrophages become the dominant white cell type (Atherton & Born, 1972) although within 24 h of injury the macrophages are well mobilised. Macrophages normally circulate in the blood as monocytes (mononuclear phagocytes) from which they migrate and enlarge following injury. They engulf and ingest large particulate matter and can coalesce to form a giant cell (macrophage polykaryon).

Polymorphonuclears, monocytes and lymphocytes, and PLT have all been shown to have a role in the inflammatory process. They can been collectively termed inflammatory cells. The roles of the phagocytic cells in acute inflammation are to identify, attack, ingest and dispose of foreign biological or other particulate matter. They can also generate and release inflammatory chemical mediators, such as PGs and cytokines. The PMNs are, however, end cells; after they accomplish

phagocytosis they are degraded or die at the inflammatory site. The role of the mononuclear phagocyte clearly is to cope with the removal of debris following the death of these PMNs (Hurley, 1978). The phagotocytic process is thought to release sufficient hydrolytic enzymes to cause damage to host tissues (Henson *et al.*, 1992). Lymphocytes also play a role in the inflammatory process. Apart from neutrophils, lymphocytes are present in inflamed exudate in relatively large quantities following the inflammatory stimulus (Higgins *et al.*, 1987). In the immunoinflammatory process, immunised T-lymphocytes activate macrophages rapidly to respond to a repeat attack by the process known as cell mediated immunity while B-lymphocytes are responsible for the production of antibodies (Welch & Winfield, 1992).

Circulating leukocytes do not adhere to vascular tissues in normal physiological conditions. For a cell to arrive at a site of inflammation it needs firstly to move from the lumen of the blood vessel (margination), then to adhere to the vascular endothelium, next to move through the vascular wall (diapedesis) and its associated tissue, and finally to move to the extravascular site of inflammation (chemotaxis). This overall process of migration may thus be divided into phases known respectively as margination, adherence, diapedesis, and chemotaxis. Recent studies have illustrated that the chemical substances released from microvasculature play important roles in cell adhesion and recruitment. A number of adhesion molecules have been identified, including E-selectin, P-selectin, L-selectin, intracellular adhesion molecule 1(ICAM-1), vascular cell adhesion molecule 1 (VCAM-1), and leukocyte integrins in the adhesion of leukocytes, PLT, and endothelium at sites of inflammation. Activated endothelial cells play a key role in "targeting" circulating cells to inflammatory sites by expression of various adhesion molecules, which serve as the receptors for cell adhesion (Gallin *et al.*, 1992; Bevilacqua & Nelson, 1993; Cronstein & Weissman, 1993). A number of chemical mediators are involved in regulation of inflammatory cellular recruitment and these will be discussed in section 1-3.

1. 2.3. Inflammatory mediators

It is now generally accepted that the pathophysiological changes in the inflammatory process are brought about or regulated by chemical mediators acting locally within the affected tissues. These biologically active substances are many in number and diverse in structure. The identification of inflammatory mediators, and their roles and regulation has become one of the

most interesting research areas. In 1918, Dale and Richards identified certain specific characteristics which a substance required to be defined as a mediator of a biological event. Vane (1972) refined the criteria for defining an inflammatory mediator as follows: it should be demonstrably present at the site of inflammation and absent when the reaction subsides; it should produce one or more of the cardinal signs of inflammation if introduced into normal tissue; antagonists of the substance should have antiinflammatory effects; the inflammatory response in tissues should be inhibited following depletion of the substance or inhibition of its synthesis; and administration of antagonists of the inactivation of the substance should increase the effects of the stimulus. Based on these criteria, a number of classical methods were developed to examine the roles and regulation of the inflammatory mediators. Recently with the development of cellular and molecular biology, a number of isoenzyemes responsible for the generation of inflammatory mediators have been identified and these provide a good basis for understanding of the roles of the mediators in both physiological and pathological processes. The use of antisense oligonucleotides provide an method of differentially regulating the isoenzymes and enzymes *in vitro.* Gene knockout techniques allow the investigator to remove a particular gene which encodes an inflammatory mediator in experimental animals and thus use the animal and its cells to unequivocally define the roles of the inflammatory mediator. A number of new inflammatory mediators and receptors have been identified so that the family of inflammatory mediators has become larger and larger in number (Gallin *et al.*, 1992; Wei *et al.*, 1995; Williams and DuBois, 1996).

Mediators known to contribute importantly to the inflammatory process include cytokines, eicosanoids, PLT-activating factor, complement and the kinin systems, histamine and 5-HT. Selectins, adherent molecules, sensory neuropeptides, substance P, calcitonin gene-related peptide, NO and pro-inflammatory neurokinins have been recently shown to significantly contribute to the inflammatory process. Most of the above substances are like a double edged sword acting in the body. They are involved in physiological processes to maintain homeostasis in the body, but their excessive generation can lead to pathological processes—inflammation (Gallin *et al.*, 1992).

Histamine

Histamine is one of the earliest identified mediators of the inflammatory process. This simple amine [4-(2-aminoethyl) imidazole] is derived from the amino acid histidine by decarboxylation and is stored as a complex with heparin in mast cells and basophils. It has also been found in PLT, nervous tissues, lungs, skin, intestinal mucosa and in the parietal region of the stomach (Levy 1974). Histamine is readily released from its granules by many stimuli which may be physical, chemical, pharmacological or immunological. Its release can be regulated by intracellular levels of cAMP or cGMP and by the disruption of microtubule (Atkinson *et al.*, 1992). Histamine causes dilatation of the terminal arterioles and increases the vascular permeability of post-capillary venules by initiating the contraction of the vascular endothelial cells. This effect is only transient however, and contributes only to the early stages of an acute inflammatory response. Thus H_1 histamine-receptor antagonists are generally only useful for the treatment of vascular events in the early transient phase of inflammation. The effect of histamine on vascular permeability is potentiated by COX products of arachidonic acid metabolism (Williams & Morley, 1973).

Histamine has effects on inflammatory cells. It was shown to inhibit the release of lysosomal enzymes from human PMN (Zurier *et al.*, 1974) and to inhibit the chemotaxis of human PMN and basophils (Hill *et al.*, 1975). The chemotactic response of eosinophils, however, is selectively enhanced by histamine, although high concentrations induce desensitisation of these cells to chemoattractants (Clark *et al.*, 1975).

Recent studies have suggested that mast cells evoked leukocyte-endothelial cell interaction by release of histamine. Thromboxane A_2 rapidly induces mast cell degranulation, leading to release and accumulation of histamine in the perivenular compartment. The mast cell-derived histamine appears to mediate at least part of the leukocyte-endothelial and PLT-leukocyte adhesion by engaging H_1-receptors on endothelial cells and PLT, respectively, to increase the expression of P-selectin. Activation of the leukocyte adhesion glycoprotein CD11/CD18 and its subsequent interaction with constitutively expressed ICAM-1 also contributes to the leukocyte recruitment elicited by C difficile toxin A (Kubes & Granger, 1996). The interaction of leukocyte-endothelial

cells leads to the generation of a broad spectrum of other inflammatory mediators, such as the eicosanoids.

5-Hydroxytryptamine

5-Hydroxytryptamine is stored in PLT and also in mast cells of rats and mice. The main storage site in mammals is the enterochromaffin cell of the gastro-intestinal tract, which contains 90 % of the total 5-HT present in the body. 5-Hydroxytryptamine is considerably more potent than histamine in increasing vascular permeability. In experimental animals, the early stages of a carrageenan-induced paw oedema have been shown to be dependent on a simultaneous release of both histamine and 5-HT. Treatment with histamine and 5-HT antagonists (mepyramine and methysergide), or depletion of both agents with compound 48/80 prior to carrageenan injection, effectively suppresses the early development of the oedema (Crunkhorn & Meacock, 1971; Di Rosa *et al.*, 1971). Recent studies showed that 5-HT potentiated pain produced by other inflammatory mediators and activation of 5-HT_2A receptors produced the same results (Abbott *et al.*, 1996). However its involvement in acute inflammation in man seems to be minor, although it may have a more important place in inflammatory conditions of the bowel.

Complement

Complement is composed of 20 plasma proteins. Complements can be activated by a variety of stimuli and are involved in maintaining host defences and mediating inflammation and tissue injury. The most important products of complement in host defensive and inflammatory process include large fragments of C_3 (e.g., C_{3b}, C_{3bi}) with opsonic activity, as well as low-molecular-weight peptides (derived from C_3 and C_5) that exhibit anaphylatoxin activity and that directly stimulate leukocytes (Goldstein, 1992).

Kinins

Kinins, including the peptides bradykinin and kallidin, are generated in the inflamed tissues by the stimulation of a variety of factors including tissue damage, allergic reactions, viral infections and other inflammatory events (Wachtfogel *et al.*, 1993). These peptides are autacoids that act

locally to produce pain, vasodilatation, increased vascular permeability and the synthesis of PGs. Thus they comprise a subset of the large number of mediators that contribute to the inflammatory response (Sharma & Mohsin, 1990; Dray & Perkins, 1993; Geppetti, 1993; Lerner, 1994).

Cytokines

Cytokines are a heterogeneous group of substances generated by cells in response to antigenic or mitogenic stimulation. They are identified by virtue of their cellular origin and by the effects they produce on target cells such as lymphocytes and macrophages. Cytokines appear to fall into three groups: cytokines that act primarily as positive or negative growth factors for a variety of cells (IL-1, IL-3, IL-4, IL-5, IL-7, IL-10, IL-11 and IL-12), cytokines that possess pro-inflammatory properties (TNF-α/β, IL-1α/β, IL-6, IFN-α/γ, IL-8, macrophage inhibitory protein-1) and cytokines that act as antiinflammatory factors [IL-1ra (a competitive antagonist of IL-1), soluble IL-1 receptors, TNF-α and IL-binding proteins] (Van Miert, 1994).

The involvement of IL-1 and TNF in inflammation has been well documented. Both IL-1 and TNF are derived from mononuclear cells and macrophages (as well as other cell types) and induce expression of numerous genes to promote the synthesis of a variety of proteins that contribute to inflammatory events. Interleukin-1 and TNF produce many of the same inflammatory responses, which include induction of fever, sleep, and anorexia; mobilisation and activation of PMN; induction of COX and LOX enzymes; increase in adhesion molecule expression; activation of B-cells, T-cells and natural killer cells; and stimulation of production of other cytokines. Other actions of these agents likely contribute to the fibrosis and tissue degeneration of the chronic proliferative phase of inflammation; stimulation of fibroblast proliferation, induction of collagenase, and activation of osteoblasts and osteoclasts. Interleukin-1 and TNF are considered principal mediators of the biological response to bacterial lipopolysaccharides and many other infectious stimuli and may work in concert with each other and with growth factors and other cytokines, such as IL-8 and related chemotactic cytokines (chemokines), which can promote neutrophil infiltration and activation (Dinarello, 1992).

Pro-inflammatory cytokines may cause acute phase responses in ruminants (including goats). It has been reported that IL-1 and TNF-α caused fever, tachycardia, anorexia, forestomach

hypomotility, neutropaenia, neutrophilia, lymphopaenia, hyperglycaemia, hypoglycaemia, hypoferraemia, hypozincaemia, cachexia, increase in acute-phase proteins and decrease in P450, and these clinical effects, haematological and blood biochemical changes resembled those observed after i.v. LPS. In addition, the time of onset of the effects after injection of these cytokines was significantly shorter than after LPS, which is in accordance with the well-documented roles of IL-1 and TNF-α as pivotal mediators of LPS toxicity (Van Miert, 1994).

Nitric Oxide

Nitric oxide is generated during the metabolism of L-arginine by the NADPH-dependent enzyme, NOS. In macrophages, NO is an intermediate in the production of both nitrate (NO^{-3}) and nitrite (NO^{-2}). There are at least two isotypes of NOS. One is constitutive, Ca^{2+}/calmodulin-dependent and is expressed mainly in the brain, PLT and endothelial cells. The other is inducible NOS which is Ca^{2+}/calmodulin-independent, existing in a variety of cell types, including macrophages, neutrophils and smooth muscle. Its expression can be induced by cytokines and bacterial products. (Liew & Cox, 1991; Moncada *et al.*, 1991).

Nitric oxide is the first gas known to act as a biological mediator and plays important roles in a number of homeostatic processes and host defence mechanisms. Nitric oxide participates in intercellular signalling when produced in small amounts by constitutive NOS in neurons and endothelial cells. However, immunologic and inflammatory stimuli induce the expression of iNOS to produce much larger amounts of NO over longer periods. In the latter circumstances, NO can exert cytostatic or cytotoxic effects against microbes, tumour cells, macrophages, and lymphocytes, as well as β cells in the pancreas (Moncada *et al.*, 1991). At the same time, NO can suppress the proliferation of T cells and the emigration of neutrophils (Kubes *et al.*, 1991).

The mechanisms by which NO promotes tissue damage have not been established. The targets of destruction by NO are non-specific and appear to involve both reactions with Fe-S groups, leading to inactivation of enzymes in the mitochondrial electron transport chain, and at high concentrations, inhibition of DNA replication (Nathan & Hibbs, 1991) and the induction of apoptosis (Sarih *et al.,* 1993). Other effects of NO, such as increase in permeability of the microvasculature, inhibition of suppressor T cells or antiproteases (McCartney-Francis *et al.,*

1993), and induction of the expression of inflammatory cytokines (Ianaro *et al.*, 1994) which induce the expression of other inflammatory mediators, might also have a role.

A role for NO has been demonstrated in acute inflammation. In rats, NO is involved in the acute inflammatory response following footpad injection of carrageenan (Lalenti *et al.*, 1992) or the topical application of mustard (Lippe *et al.*, 1993). It was also reported that NO was directly or indirectly linked to immune complex-induced tissue injury (Mulligan *et al.*, 1991). Wei *et el.* (1995) have used iNOS knockout mice to confirmed the roles of NO in inflammatory and immune responses. Their experiment demonstrated that wild-type and heterozygous mice are highly resistant to the protozoal parasite *Leishmania major* while the mutant mice are uniformly susceptible, the infected mutant mice developed a significantly stronger Th1 type of immune response than the wild-type or heterozygous mice, and the mutant mice also showed a reduced non-specific inflammatory response to carrageenan induction, and were resistant to LPS induced mortality. In most of the studies, the pathology was significantly reduced by inhibiting NO synthesis *in vivo* by treating the animals with L-NMMA while D-NMMA did not show any inhibitory effects.

Nitric oxide also plays a role in chronic inflammation. Adjuvant arthritis in rats is a model of chronic inflammation characterised by T cell- and monocyte- mediated immune response, and erosive destruction of the subchondral and periarticular bone and cartilage. The development of arthritis was accompanied by induction of iNOS and production of NO both in synovial tissue and blood mononuclear cells. The inhibition of NO synthase reduced the accumulation of inflammatory leukocytes and erosion of the joint (Lalenti *et al.*, 1993). In man, serum from patients with rheumatoid arthritis or osteoarthritis contains significantly higher levels of nitrite compared to that of matched controls (Farell *et al*, 1992).

Selectins

Recent studies have determined that a variety of cell adhesion molecules within the microvasculature play an important role in recruitment of inflammatory cells, especially the adhesion of leukocytes, PLT and endothelium at sites of inflammation. These includes selectins, intracellular adhesion molecule 1, vascular cell adhesion molecule 1, and leukocyte

integrins. Of these cell adhesion molecules, the selectin family is particularly important and has been studied in detail (Bevilacqua & Nelson, 1993). They have also been grouped as inflammatory mediators (Schlag & Redl, 1996).

Selectins are a group of molecules present on the surface of leukocytes, PLT and endothelium. Each molecule contains an NH_2 terminal lectin-like domain, an epidermal growth factor repeat and a discrete number of molecules similar to those found in certain complement binding proteins. Three types of selectins have been identified, L-selectin, P-selectin and E-selectin (Bevilacqua & Nelson, 1993). Their expression varies among the different cell types involved in the inflammatory response. Only L-selectin is constitutively expressed at the cell surface. The other two selectins are inducibly expressed. E-selectin is expressed in a restricted fashion in endothelial cells and is enhanced at sites of inflammation. P-selectin is expressed predominantly on PLT and endothelial cells and is enhanced by cytokines. L-selectin, in contrast, is a receptor for P-selectin, and it is expressed on leukocytes and is shed when these cells are activated. The enhanced selectins on resident cells can recognise and interact with the cell surface glycoprotein and the carbohydrate on circulating cells to form selectin-carbohydrate ligands and thus lead to recruitment of inflammatory cells. Although this suggests that selectins on the cell surface act like a receptor, recent reports have illustrated that soluble forms of P-selectin and L-selectin act as a functional circulating protein present at detectable concentrations (P-selectin, 0.1-0.3 μg/ml) (Dunop *et al.*, 1992) or relatively high concentrations (L-selectin, about 1.5 μg/ml) (Scheiffenbaum *et al.*, 1992) in normal human plasma. This indicates that soluble forms of selectins modulate inflammatory responses.

Selectins may act as a new therapeutic target for antiinflammatory drug development. From the perspective of invading microorganisms, the host presents a formidable array of defences. Central to this process is the host's ability to recruit leukocytes to specific sites in the body where they are most needed. Leukocyte recruitment involves an orchestration of soluble mediators (e.g. cytokines) and cell-surface molecules that results in focal leukocyte attachment to the vessel wall and extravasation. The importance of the selectins in this process is now widely accepted. On the other hand, the accumulation and degeneration of inflammatory cells at the affected sites can cause tissue damage. Thus the blockage of selectin-carbohydrate interaction may help the host to recover from the inflammation (Rao *et al.*, 1994).

Platelet-activating factor

When PLA_2 catalyses the release of arachidonic acid from lipids for the synthesis of eicosanoids, it also leads to the generation of modified phospholipids, importantly represented by PAF. Two metabolic steps are involved in the biosynthesis of PAF: the action of a PLA_2 on choline-containing membrane alkyl-ether lipids results in the production of lyso-PAF acether and acetylation of the lyso compound by an acetyltransferase yields the biologically active molecule. Platelet-activating factor is one of the potent pro-inflammatory mediators released from and acting on most cells that participate in the inflammatory response, including PLT, neutrophils, monocytes, mast cells, eosinophils and vascular endothelial cells. It induces the aggregation of PMNs and also promotes their aggregation and generation of lysosomal enzymes and eicosanoids and initiates the release of oxygen radicals. It also acts as an endogenous mediator of activation in a number of cell types (McDonald *et al.*, 1994). It mediates IL-1-induced leukocyte extravasation across rat mesenteric microvessels (Nourshargh *et al.*, 1995). Platelet-activating factor is a potent vasodilator, and it lowers peripheral vascular resistance and systemic blood pressure and induces intravascular aggregation of PLT when injected i.v. in dogs (Sybertz *et al.*, 1985). Intradermal injection of PAF duplicates many of the signs and symptoms of inflammation, including increased vascular permeability, hyperalgesia, edema and infiltration of neutrophils, and inhaled PAF increases the infiltration of eosinophils into the airways.

Eicosanoids

The families of PGs, LTs, TXs and related compounds are called eicosanoids because they are derived from 20-carbon essential fatty acids, most notably arachidonic acid by COX and LOX enzyme systems. These compounds have a vital role in the initiation and maintenance of several components of the inflammatory process. Arachidonate is esterified to the phospholipids of cell membranes or other complex lipids. Since the concentration of free arachidonate in the cells is very low, the biosynthesis of eicosanoids depends primarily upon its availability to the eicosanoid-synthesizing enzymes; this results from its release from cellular stores of lipid by acyl hydrolases, especially PLA_2 and in PLT, by diacyglycerol lipase (Prescott & Majerus, 1983). Expression of PLA_2 can be induced by widely divergent physical, chemical and hormonal

stimuli. At least two isotypes of PLA_2 have been identified in mammalian cells, $cPLA_2$ and $sPLA_2$. There is increasing evidence that $cPLA_2$ generates an arachidonic acid pool used by defensive mechanism and $sPLA_2$ generates arachidonic acid for physiologic uses (Kawada *et al.*, 1995; Reddy & Herschman, 1996). Once released, a portion of the arachidonate is metabolised rapidly to oxygenated products by several distinct enzymes, including COX and LOXs, to form different groups of eicosanoids (Fig. 1.3.).

a) Cyclooxygenase pathway

The COX pathway leads to the formation of PGs and TXs. A ubiquitous complex of microsomal enzymes are involved in these synthetic processes in a stepwise manner. Cyclooxygenase also called PG endoperoxide synthase (PGHS), is the first enzyme in the pathway leading from arachidonic acid to PGI_2, PGs and TXs. Increasingly biological data have shown that mammalian cells contain two isoforms of COX, termed COX-1 and COX-2. Cyclooxygenase-1 is a constitutive enzyme, generating PGs to modulate physiological processes whereas COX-2 is an inducible enzyme expressed following induction with stimuli, including certain serum factors, cytokines and growth factors. Cyclooxygenase-2 is thought to be responsible for inflammation and its expression can be inhibited by glucocorticoids (Kujubu *et al.*, 1991; Xie *et. al.*, 1991; O'Banion *et al.*, 1992; Sirois *et al.*, 1992; DeWitt & Meade 1993; Jones *et al.*, 1993; Herschman, 1994). Phospholipase A_2 releases arachidonic acid to provide substrate for COX. Previous studies have also shown that COX-1 is linked to $sPLA_2$ and uses the arachidonic acid pool generated by $sPLA_2$. Cyclooxygenase-2 is associated with $cPLA_2$ and uses the arachidonic acid pool generated by $cPLA_2$ (Kawada *et al.* 1995). The COXs have two distinct activities: an endoperoxide synthase activity that oxygenates and cyclizes the unesterified precursor fatty acid to form the PGG, and a peroxidase activity that converts PGG to PGH. Cyclic endoperoxide and PGH are chemically unstable, but they can be transformed enzymatically into a variety of products, including PGI, TXA, PGEs, PGFs, or PGD (in the mast cells). (Fig 1.3.).

The endoperoxide PGH_2 is also converted into two unstable and highly active compounds. Thromboxane A_2 is formed by TX synthase; TXA_2 breaks down nonenzymatically ($t_{\frac{1}{2}}$ = 30 seconds) into the stable, but inactive, TXB_2. Prostacyclin is formed from PGH_2 by PGI_2

synthase; it is hydrolysed nonenzymatically ($t_{\frac{1}{2}}$ = 3 min) to the inactive 6-keto-$PGF_{1\alpha}$. The activity of COX in this pathway can be estimated by determination of their stable metabolites.

It has been reported that PGE_2 is a predominant PG in carrageenan-induced inflammatory exudate. Willis (1969) developed an acute inflammatory model by intraperitoneal injection of carrageenan in rats and first illustrated the presence of PGE_2 in the inflammatory exudate. A further study by Higgs *et al.* (1983) confirmed these results and found that detectable concentrations of TXB_2, 6-keto-$PGF_{1\alpha}$ were also present in acute inflammatory exudate but PGE_2 was predominant. These findings were also confirmed in other animal species. Higgins and Lees (1984a) used a subcutaneous tissue-cage acute inflammatory model in ponies to demonstrate that following the injection of carrageenan into the cages the concentrations of PGE_2, 6-keto-$PGF_{1\alpha}$ and TXB_2 all temporally increased in the inflammatory exudate and reached a peak at 6-12 h. The maximal concentration for PGE_2 was 197.0 ± 64.9 ng/ml while the maximal concentrations for 6-keto-$PGF_{1\alpha}$ and TXB_2 were 18.9 ± 9.4 ng/ml and 11.5 ± 7.3 ng/ml, respectively. Similar methods were used in dogs (McKellar *et al*, 1994a, 1994b) and calves (Landoni *et al.*, 1995a, 1995b; 1996; Lees *et al.*, 1996), in which the concentrations of PGE_2 were significantly elevated in the inflammatory exudate after the injection of carrageenan into the cages.

The above experiments in domestic animals also showed that before injection of carrageenan the concentration of PGE_2 was very low or undetectable. This is in accordance with the finding that COX-2 is not expressed unless stimulated and suggests that exudate PGE_2 was predominantly generated by COX-2. A study using a rat carrageenan subcutaneous air pouch model has confirmed this hypothesis, in which selective COX-2 inhibitors (NS-398) significantly reduced exudate PGE_2 generation and subsequent inflammation without a concomitant decrease in gastric PG production (by COX-1) or development of gastric lesions (Masferrer *et al.*, 1994).

The products of the COX pathway of arachidonic acid metabolism form a group of important inflammatory mediators which substantially induce the cardinal signs of acute inflammation through their direct and indirect actions. Prostaglandin I_2 and E_2 have a prolonged vasodilatory effect which causes erythema (Williams, 1979). Although PGs do not appear to have direct effects on vascular permeability, both PGE_2 and PGI_2 markedly enhance oedema formation and

leukocyte infiltration by promoting blood flow in the inflamed region. They potentiate the increases in vascular permeability produced by histamine and bradykinin leading to increased oedema formation (Williams & Morley, 1973). The E PGs enhanced the pain-producing effects of bradykinin (Moncada *et al.*, 1974). Prostaglandins and PGI_2 sensitise the afferent nerve endings to the effects of chemical or mechanical stimuli by lowering the threshold of the nociceptors (Moncada *et al.*, 1978). The studies in our laboratory demonstrated that COX inhibitors reduced the hyperalgesia evoked by intradermal injection of carrageenan in sheep and this suggests that PGs play a role in pain perception (Welsh & Nolan, 1994b). Prostaglandins cause an increased inflammatory local temperature by raising blood flow in the affected region. The roles of PGs in inflammatory heat generation have been established in horses (Higgins *et al.*, 1987; Lees *et al.*, 1987b, 1987c), cattle (Lees *et al.*, 1996) and dogs (McKellar *et al.*, 1994a; 1994b). These studies showed that intracaveal injection of carrageenan led to an increase in skin temperature over the cages which is correlated with the increase in exudate PGE_2 generation within the cages. Administration of COX inhibitors reduced the formation of PGs and the subsequent increase in temperature. Thromboxane A_2 is a potent vasoconstrictor and a powerful inducer of PLT aggregation and of the PLT release reaction which leads to the release of a number of inflammatory mediators and the adhesion of PLT to leukocytes and endothelia to leukocytes (Moncada & Vane, 1979; Spagnuolo *et al.*, 1980).

The effects of PGs are diverse and their mechanisms of action can be explained by the existence of a number of distinct receptors. The receptors have been named according to their natural affinity and have been allocated into five main types, designated DP (PGD), FP (PGF), IP (PGI_2), TP (TXA_2), and EP (PGE) (Davies & MacIintyre, 1992). The actions of PGs have been extensively studied in PLT. The PG endoperoxides and TXA_2 stimulate the TP receptors and thereby activate PLT aggregation, a response associated with PLC. Subsequent release of intracellular Ca^{++} promotes aggregation and production of additional TXA_2 (Davies & MacIntyre, 1992).

b) Lipoxygenase pathway

As free arachidonic acid is released from the body's phospholipid "pools", its unsaturated carbon atoms are susceptible to oxygenation by various positional LOXs (Fig. 1.3.). These carbon atoms

include those at the 5, 11, 12, and 15 positions and a variety of products are possible, including mono and di HPETEs. The HPETEs are unstable and may be further converted to their corresponding hydroxy fatty acids, HETEs, by a peroxidase or nonenzymatically (Fig. 1.3.). Lipoxygenases differ in their specificity for placing the hydroxy group, and tissues differ in the LOXs that they contain. For example, PLT have only 12-LOX and synthesise 12-HPETE, whereas leukocytes contain both 5-LOX and 12-LOX and produce both 5-HPETE and 12-HPETE.

The formation of LTA_4 by 5-LOX perhaps is the most important of these steps, since it leads to the formation of LTB_4 (5,12-diHETE) by one pathway and LTC_4, LTD_4 and LTE_4 by another. Leukotriene B_4 is a dihydroxy acid formed from LTA_4 through the action of LTA_4 hydrolase. Leukotriene B_4 can undergo further oxidation at the 20 carbon atom to less active metabolites (Henderson, 1991). Leukotriene A_4 may be conjugated with glutathione by LTC_4 synthase to form LTC_4. Leukotriene D_4 is produced by the removal of glutamic acid from LTC_4, and LTE_4 results from the subsequent cleavage of glycine; the reincorporation of glutamic acid yields a γ-glutamylcysteinyl derivative called LTF_4 (Piper, 1984; Samuelsson *et al.*, 1987).

The metabolites of arachidonic acid formed via LOX pathway comprise a group of important inflammatory mediators. 12-HETE is chemotactic for PMN leukocytes (Turner *et al.*, 1975). Leukotrine C_4, LTD_4 and LTE_4 modulate vasoconstriction and vascular permeability (William, 1994), and the mixture of them is now believed to be "slow reacting substance" of anaphylaxis (SRS-A). Leukotriene B_4 has been shown to be an extremely potent chemotactic agent for many cell types but notably for PMNs in many experimental animal species (Ford-Hutchinson *et al.*, 1980) and horses (Sedgwick *et al.*, 1987). Leukotriene B_4 plays a role in induction of neutrophil-endothelial cell adhesion, neutrophil degranulation, lysosomal enzyme release and in immune modulation by upregulation of cytokines (William, 1994). Leukotriene B_4 may be an important mediator of inflammatory pain. Injecting LTB_4 into rat paw results in a prolonged, neutrophil-dependent hyperalgesic reaction, which is associated with a sustained reduction in the nociceptive pressure threshold (Levine *et al.*, 1984).

The metabolism of arachidonic acid via the LOX pathway has been studied in several domestic animal species, including horses (Higgins & Lees 1984b; Lees *et al.*, 1987c), dogs (McKellar *et*

al., 1994a, 1994b) and calves (Landoni *et al.,* 1995a, 1995b). These studies used subcutaneous tissue cage acute inflammatory models and indicated that following the injection of carrageenan into the cages, the concentrations of 12-HETE or LTB_4 increased and was accompanied by an increase in accumulation of WBC in the inflammatory exudate. The studies in horses and calves also demonstrated that the inhibition of COX by NSAIDs led to the increase in LOX products due to a shift of metabolic substrate and that the inhibition of COX did not affect exudate WBC number significantly. Previous studies in man reported increased concentrations of LTs, especially LTB_4 in the affected sites or circulating blood in a number of inflammatory diseases, including rheumatoid arthritis, psoriasis and inflammatory bowel disease (William, 1994). The presence of LOX-derived eicosanoids in inflamed tissues could represent a local control mechanism for the recruitment of inflammatory leukocytes. It is believed that LOX inhibitors could be useful therapeutic agents and inhibitors of LOX and LT receptors are under development.

1.3. Pharmacology of NSAIDs

1.3.1. Pharmacodynamics of NSAIDs

Nonsteroidal antiinflammatory drugs have been widely used in both human and veterinary medicine for centuries for their antiinflammatory, antipyretic, and analgesic effects. Although they had been shown to inhibit a wide variety of reactions in a number of experiments, no convincing relationship could be established with their underlying effects until 1971, when Vane and his colleagues demonstrated that low concentrations of aspirin and indomethacin inhibited the enzymatic production of PGs. An understanding of the roles of PGs in the inflammatory process has supported the hypothesis that inhibition of the biosynthesis of these autacoids could explain a number of the clinical actions of the drugs (Feldberg & Saxena, 1971a, 1971b). Numerous subsequent observations have confirmed this hypothesis. Studies in veterinary medicine soon followed and in the early 1980s, Lees and Higgins at the Royal Veterinary College conducted a number of experiments to investigate the roles of PGs and mode of action of NSAIDs using sponge and tissue-cage acute inflammatory model in ponies (Higgins *et al.*, 1984; Higgins & Lees, 1984a 1984b, 1984c; Lees & Higgins 1984; Lees *et al.*, 1987a, 1987b, 1987c). Their studies confirmed the above hypothesis in domestic animal species and stimulated further

studies of inflammatory mediators and NSAIDs in farm animal species. A number of investigations have been carried out in different animal species, including dogs (McKellar *et al.*, 1989, 1994a, 1994b) and calves (Landoni *et al.*, 1995a, 1995b, 1996; Lees *et al.*, 1996). These experiments established the relationship between inhibition of COX and the antiinflammatory effects of NSAIDs. In recent years a large amount of evidence have emerged suggesting that NSAIDs may have antiinflammatory and other effects which are independent of COX inhibition (McCormack & Brune, 1991; Twomey & Dale, 1992). Landoni *et al.* (1995a, 1995b, 1996) reported that FM, KPF and tolfenamic acid effectively inhibited β-glucuronidase activity and bradykinin-induced swelling in calves in addition to the inhibition of COX. Other actions of NSAIDs have been reported but their contribution to the therapeutic effects has not been estimated (Abramson & Weissman, 1989; Vane, 1994).

It is now believed that NSAIDs produce their principal therapeutic and their undesirable side effects by inhibition of COX and thus by blocking the formation of COX-derived eicosanoids. Since the identification of COX-1 and COX-2, interest has been focused on these isoenzymes (Kujubu *et al.*, 1991; Xie *et. al.*, 1991; O'Banion *et al.*, 1992; Sirois *et al.*, 1992; DeWitt & Meade 1993; Jones *et al.*, 1993; Herschman, 1994; Vane, 1994; Vane & Botting, 1995). The inhibition of COX-2 is thought to produce, at least in part, the antipyretic, analgesic, and antiinflammatory effects, but the simultaneous inhibition of COX-1 may be largely responsible for unwanted side effects, particularly those leading to gastric ulcers, that result from decreased PG formation. Thus COX-2 is probably the therapeutic target of NSAIDs. Most currently used NSAIDs non-selectively inhibit the COX-1 and COX-2 isoforms or have modest selectivity for the constitutive COX-1 isoform. This means that they produce both therapeutic effects and unwanted effects simultaneously. An ideal NSAID should possesses good inhibitory effect for COX-2 and less or no inhibition of COX-1. Cyclooxygenase-2 selective NSAIDs are now under development and it is anticipated that they will be better NSAIDs for antiinflammatory therapy (Vane, 1994; Vane & Botting, 1995; Williams & DuBois, 1996). It has been reported that the selective COX-2 inhibitor, NS-398, effectively inhibited carrageenan-induced inflammation without a concomitant decrease in gastric PG synthesis or the development of gastric lesion in an air pouch inflammatory model in the rats (Masferrer *et al.*, 1994).

The inhibition of COX by NSAIDs may be reversible or irreversible. Platelets are especially susceptible to aspirin-mediated irreversible inactivation of COX because they have little or no capacity for protein biosynthesis and thus cannot regenerate the COX enzyme. A single clinical dose of aspirin (19 mg/kg) given to the horse abolished the serum PLT TXB_2 generation for 7 days and 74 % inhibition was still present after 24 days (Lees *et al.*, 1987a). The other NSAIDs currently used in veterinary practice, including FM, PBZ, KPF, tolfenamic acid etc., are competitive and reversible inhibitors. They inhibit PLT COX (COX-1) and carrageenan-induced exudate COX (COX-2) reversibly in a number of tested animal species including horses (Lees *et al.*, 1987a, 1987b; 1987c), dogs (McKellar *et al.*, 1989, 1994b,) and calves (Lees *et al.*, 1991c; Landoni *et al.*, 1995a, 1995b, 1996).

The mechanisms for the inhibition of COX isoenzymes are not fully clear. In the mice, COX-1 shares about 62 % amino acid identity with COX-2 (Rosen *et al.*, 1989; Sirosis & Richards, 1992). They express differentially in physiological and pathological processes. Aspirin acetylates serine 530 of COX-1 to prevent the binding of arachidonic acid to the active site of the enzyme and thus the ability of the enzyme to generate PGs. In COX-2, aspirin acetylates a homologous serine at position 516. Although covalent modification of COX-2 by aspirin also blocks the COX activity of this isoform, it diverts the metabolism leading to the synthesis of 15-HETE (Lecomte *et al.*, 1994; O'Neil *et al.*, 1994). 15-hydroxyeicosatetraenoic acid may be an active inflammatory mediator and this may increase the side effects of aspirin. Another study using sheep COX-1 reported that arginine 120 of COX-1 was required for the inhibition by NSAIDs containing a carboxylic acid moiety (Mancini *et al.*, 1995).

The inhibition of COX activity by NSAIDs leads to the relief of inflammatory symptoms. Nonsteroidal antiinflammatory drugs are usually classified as mild analgesics. In some forms of postoperative and inflammatory pain which are caused by PGs, the NSAIDs can be superior to other analgesics. A good example is FM which can mask the pain of colitis and laminitis in horses. Nonsteroidal antiinflammatory drugs are widely used antipyretics. Regulation of body temperature requires a delicate balance between the production and loss of heat; the hypothalamus regulates the set point at which body temperature is maintained (Saper & Breder, 1994). During inflammation, the enhanced formation of cytokines, such as IL-1β, IL--6, IFN α and β, and TNFα increase the synthesis of PGE_2 in circumventricular organs in and near to the

preoptic hypothalamic area. The PGE_2 thus formed increases cyclic AMP activity and triggers the hypothalamus to elevate body temperature by promoting increases in heat generation and decreases in heat loss. Nonsteroidal antiinflammatory drugs suppress this response by inhibiting the synthesis of PGE_2 (Dascombe, 1985). Prostaglandins cause increased temperature in inflammatory foci by dilating the blood vessels to enhance the blood flow in the affected site. Nonsteroidal antiinflammatory drugs inhibit PG generation and thus prevent the temperature elevation in the inflammatory sites. This has been demonstrated in a number of animal species, including horses (Lees *et al.*, 1987b, 1987c) and dogs (McKellar *et al.*, 1994a, 1994b). However, NSAIDs do not affect the hyperalgesia, pain or fever caused by injection of PG, consistent with the notion that the effects of these agents are due to inhibition of PG synthesis (Davies & MacIntyre, 1992).

For some of the NSAIDs the relationship between COX inhibition, and analgesic and antipyretic effects is not significant. For example, CPF and PRT are weak COX inhibitors but they are widely used as analgesics and antipyretics. The mode of action of these NSAIDs is unknown. Paracetamol has been shown to inhibit COX only in an environment that is low in peroxide. Inflammatory sites usually contain increased concentrations of peroxides generated by leukocytes and this may prevent PRT from inhibiting COX. However in the central nervous sites, such as hypothalamus, low concentrations of peroxide exist and they may allow PRT to inhibit COX (Marshall *et al.*, 1987; Hanel & Lands, 1982). This may in part explain the mechanism of this NSAID.

Arachidonic acid also can be converted, via 12-LOX, to 12-HPETE or, via the 5-LOX, to a variety of leukotrienes. Nonsteroidal antiinflammatory drugs generally do not inhibit LOXs. As shown in Fig. 1.3., inhibition of COX may lead to the accumulation of arachidonic acid which can be used by LOXs and thus the increased LOX derived eicosanoids (Kitchen *et al.*, 1985; Sedgwick *et al.*, 1987). This may also contribute to the side effect of NSAIDs. Dual inhibitors for COX and LOX are under development (Cunningham & Lees, 1994) and they may be expected to be more effective NSAIDs for antiinflammatory therapy.

1.3.2. Pharmacokinetic characteristics of NSAIDs

Nonsteroidal antiinflammatory drugs exert their principal therapeutic effects in inflammatory sites by inhibition of COX-2 and produce their side effects in the gastrointestinal system, PLT and kidney by blockage of COX-1, which leads to gastrointestinal ulceration, disorders of PLT aggregation and renal function. For the irreversible inhibitors of COX (such as aspirin), the duration of effects is determined by the rate of synthesis of new COX enzymes. The other NSAIDs used in veterinary medicine are competitive and reversible inhibitor of COX enzymes and their effects are primarily related to the PK clearance of the drugs from the effect sites. Thus PK disposition of those NSAIDs are of critical importance to the understanding of their therapeutic benefits and risks.

A number of studies have shown that a range of NSAIDs used extensively in veterinary medicine (PBZ, aspirin, FM, naproxen and CPF) exhibit marked species differences in PK parameters so that the data can not be transposed between species (McKellar *et al.*, 1990, 1994a; Welsh *et al.*, 1993; Cunningham & Lees, 1994; Landoni *et al.*, 1995a, 1995b, 1996). In general, the elimination rates of NSAIDs in ruminants such as calves and sheep are slower than in monogastric animal species, such as horses and dogs.

Increasing attention has been paid to the chirality of NSAIDs. Of particular interest are the newer arylpropionic acid derivatives (CPF and KPF). These drugs are supplied as racemixtures. However in the chiral environment of the body, the proportion of enantiomers formed from a racemate can vary in different animal species, and both the PK and PD behaviour can differ between the enantiomers. Previous studies showed that when rac-CPF was given, the R(-) enantiomer was predominant in all species investigated, including horses (Lees *et al.*, 1991a), dogs (McKellar *et al.*, 1994a), cats, goats, pigs and calves (Delatour *et al.*, 1993; Lees *et al.*, 1996). For KPF both antipodes show similar profiles in man, S(+)KPF is predominant in dogs, and R(-)KPF predominates in sheep (Delatour *et al.*, 1993). Generally for NSAIDs, S(+) enantiomer is the eutomer which is responsible for the pharmacological effects while R(-) enantiomer is the distomer which is an inactive antipode. Therefore the pharmacological effects produced by a racemic drug represent the combined effects of the enantiomers formed in the particular animal species. When a dosage schedule is made, particular attention should be paid to

the enantiomorphism of the drug in the targeted animal species. It has been suggested that non-enantioselective studies should in future be avoided and racemic mixtures of chiral drugs should be considered as combinations of two or more distinct compounds with likely differences in PK, PD and toxicological profiles (Landoni & Lees 1996).

An attractive PK property of many of the drugs used as antiinflammatory agents is that they accumulate at sites of inflammation. With the exception of aspirin, most of the NSAIDs serve as reversible competitive inhibitors of COX activity. This means that they produce time-dependent effects in accordance with the distribution and elimination of the drugs at the sites of therapeutic effect. As organic acids, NSAIDs generally are well absorbed p.o., highly bound to plasma proteins and excreted either by glomerular filtration or by tubular secretion. Studies using a tissue-cage inflammatory model have illustrated that the drug concentrations and AUC in inflamed exudate are higher than in non-inflamed transudate, the plasma drug concentrations in plasma were initially higher and subsequently lower than in exudate and transudate following administration of NSAIDs. This has been shown for FM in horses and calves (Lees and Higgins, 1984; Lees 1989; Landoni *et al.*, 1995a), PBZ in horses (Lees & Higgins, 1986; Lees *et al.*, 1986), meloxicam in horse (Lees *et al.*, 1991b), CPF in horses and dogs (McKellar *et al.*, 1994a; Lees *et al.*, 1991a, 1994), KPF in calves (Landoni *et al.*, 1995b), tolfenamic acid in dogs and calves (McKellar *et al.*, 1994b; Landoni *et al.*, 1996). The mechanism of the high extravascular penetration of NSAIDs may be attributed to the high degree of drug binding to plasma proteins. The increased vascular permeability in inflammatory sites allows the leakage of plasma protein into the extravascular space, with the NSAIDs. Another possible reason is that the acidic property of inflammatory exudate has a special affinity to the acidic NSAIDs. Since most of the NSAIDs have a high degree of plasma protein binding and their therapeutic target, COX-2, is expressed in the inflammatory sites following the injury, exudate PK data may be more useful than plasma PK data for predicting appropriate dosage schedules of NSAIDs for clinical use.

1.4. Acute inflammatory models for testing NSAIDs in domestic animal species

Because of the differences between NSAIDs in both PDs and PKs in domestic animal species, experiments must be carried out in the target domestic animal species. When developing equine models of inflammation, Higgins and Lees proposed that the inflammatory responses should be

mild, reproducible and reversible so that the animals can be used repeatedly and cross-over studies can be undertaken (Higgins & Lees, 1984a, 1984b, 1984c; Higgins *et al.*, 1987; Lees *et al.*, 1987c). The inflammatory reaction should also be inhibited by NSAIDs in a characteristic dose-responsive fashion. Based on these requirements, two experimental exudative models of acute non-inflammation, the polyester sponge model and tissue-cage model have been developed in the ponies (Higgins *et al.*, 1987).

The tissue-cage model has now largely supplanted the other model for screening and assessing NSAIDs in veterinary domestic animal species. Firstly, the model provides a mild, reproducible and reversible inflammatory reaction which is free from uncontrolled incidental factors and which causes minimal distress to the experimental animals. Secondly, the animals can be used repeatedly so that cross-over studies can be undertaken. Thirdly, this model permits the sequential collection of inflammatory exudate for the analysis of drug concentrations, inflammatory cells and mediators so that the relationship between drug concentrations and therapeutic effects can be established. Finally, the important clinical sign of heat, can be quantified by measuring the skin temperature over the cages.

The choice of tissue cage may vary based on the size of the targeted animals. Multiperforated polypropylene practice golf balls placed in the midneck region were most appropriate for the large animal species including ponies (Higgins & Lees 1984a, 1984b, 1984c) and calves (Lees *et al.*, 1991c, 1996; Landoni *et al.*, 1995a). Each ball had an internal diameter of 41 mm and internal volume of 31 ml. Each ball had 25 holes, corresponding to about 9.3 % of total surface area, punched in it. In dogs, McKellar and colleagues (1994a, 1994b) implanted tube-like cages in the flanks and succeeded in inducing an inflammatory response and collecting exudate. The cages were 7.5 cm long with a 1.6 cm external diameter and were made from silastic tubing with plugs of silastic glued in either end and holes punched in the barrel of the tube close to each end. The cages were subcutaneously implanted using lignocaine infiltration anaesthesia and xylazine sedation. In dogs, general anaesthesia was induced. Following the implantation fibrous granulation tissue gradually grows into the cages through the holes and a large proportion of the interior of each cage is fitted. This granulation tissue is the target for inflammatory induction by irritants. The acute inflammation may be generated by intracaveal injection of carrageenan solution and maintained by a further injection 8 h later. The effective dose of carrageenan for

PGs production and leukocyte migration in this model (golf ball model) is 0.5 ml of 1% solution (Higgins & Lees 1984a, 1984c). The changes in skin temperature over the inflamed and non-inflamed cages may be quantified as a clinical indicator of the inflammatory responses.

The inflammatory processes have been intensively studied in the equine model (Higgins & Lees 1984a, 1984b, 1984c; Higgins *et al.*, 1987; Lees *et al.*, 1984; 1987b, 1987c). In the equine model leukocyte migrates into the cage in large numbers in a time dependent fashion and maximum numbers were detected at 12 h (20×10^9/L) following the injection of carrageenan. After 48 h, the number of exudate leukocytes decreased to control values (Higgins & Lees, 1984a, 1984b). Prostaglandins including PGE_2, 6-keto-$PGF_{1\alpha}$ and TXB_2 were present in the exudate and achieved maximal concentrations at 6-12 h following the carrageenan induction. However the predominant PG was PGE_2 which reached peak concentrations of about 100 ng/ml in the exudate. The total protein in exudate increased and the concentration was similar to that in plasma. Higgins and Lees (1984b) reported that LTB_4 in equine exudate was detectable and with a mean concentration of 1.7 ng/ml achieved at 8 h after carrageenan stimulation.

The carrageenan tissue cage model of acute inflammation has been used to investigate the PDs and PKs for a number of NSAIDs in different domestic animal species, at least in horses (Lees & Higgins 1984; Lees *et al.*, 1986, 1987b, 1987c, 1991c, 1991b), dogs (McKellar *et al.*, 1994a; 1994b) and calves (Landoni *et al.*, 1995a; 1995b; 1996; Lees *et al.*, 1996). The results demonstrate the expected PD and PK responses. The NSAIDs with antiinflammatory effects which inhibit exudate PGE_2 and simultaneously inhibit the increase in skin temperature over the cages. The NSAIDs with weak antiinflammatory effects do not generally inhibit exudate PGE_2 generation or the inflammatory temperature rise. No NSAID has been shown to effectively block the activity of LOX and the recruitment of leukocytes in the inflammatory foci. Most of the tested NSAIDs accumulate at the inflammatory sites and this is indicated by a larger AUC in exudate than in transudate, and slower elimination rate from exudate than from transudate and plasma.

The tissue cage model has been used to dose responsive PD and PK studies for NSAIDs. McKellar *et al.* (1994b) gave tolfenamic acid to dogs by intramuscular injection at dosage rates of 2, 4 and 8 mg/kg of body weight and obtained dose-related differences in drug concentration

in exudate and statistically different inhibitory effects for exudate PGE_2 between each treatment group.

1.5. **Antiinflammatory therapy in veterinary medicine**

Many classes of drug are used to suppress or abolish one or more of the cardinal signs of inflammation. The main antiinflammatory groups used in veterinary medicine are corticosteroids and NSAIDs.

1.5.1. Corticosteroids

The corticosteroids used in veterinary medicine include betamethasone, dexamethasone, prednisolone, flumethasone and isoflupredone. In general, corticosteroids have a wider spectrum of pharmacological activity than NSAIDs (Fig. 1.3.). Unlike NSAIDs which principally act on COX, corticosteroids inhibit both COX and LOXs. They also have inhibitory effects on other inflammatory mediators, including histamine, bradykinin, PAF, cytokines, NO, selectins and adhesion molecules. They not only inhibit vascular and cellular responses characteristic of acute inflammation but also the proliferative, self perpetuating and damaging features of the chronic inflammatory process. They are often used for the suppression of inflammatory reactions caused by infection, trauma, allergy or other causes.

Corticosteroids, such as dexamethasone, block the induction of $cPLA_2$ and thus inhibit the generation of eicosanoids (Samet *et al.,* 1995). They inhibit COX-2 in various contexts, including transcription and expression of the COX-2 gene and have less inhibitory effect on the expression COX-1 gene (Herschman, 1994).

However, corticosteroids have serious potentially toxic side-effects which limit their clinical use. This includes atrophy of the adrenal cortex, water and electrolyte retention, metabolic disturbances and susceptibility to infection associated with immunosuppression.

1.5.2. Nonsteroidal antiinflammatory drugs

1.5.2.1. Clinical uses of NSAIDs in veterinary medicine

Nonsteroidal antiinflammatory drugs are known to exert three types of pharmacological activity, analgesia, antipyrexia and antiinflammatory activity. The principal value of these drugs is to relieve pain and reduce inflammation. Currently licensed NSAIDs with antiinflammatory and analgesic indication in veterinary use in the UK include CPF (horses and dogs), FM (horses, cattle and dogs), KPF (horses, cattle, dogs and cats), meclofenamic acid (horses), meloxicam (dogs), naproxen (horses and dogs), PBZ (horses, dogs and cats) and tolfenamic acid (dogs and cats). Flunixin can be used by the i.v., i.m. and p.o. administration routes while others are used as i.v., i.m. or p.o. administration preparations.

One of most interesting effects of NSAIDs in veterinary medicine is the antiinflammatory action although they are often used as analgesics. The reason for this is that inflammation is a common process in a wide range of diseases and drugs with this effect may improve the demeanour of affected animals. In addition to their classical use for treating musculoskeletal conditions and colic in horse, they have been recently extensively used in other species and diseases. For example, FM is used for the treatment of musculoskeletal conditions, endotoxemia, and colic in horses; for musculoskeletal conditions and control of postoperative pain in dogs; and for mastitis, endotoxaemia, and bacterial and viral pneumonia in calves.

1.5.2.1. Chemical classification of NSAIDs

Nonsteroidal antiinflammatory drugs are weak organic acids which contain a heterogeneous group of compounds, often chemically unrelated. Based on the chemical structural classes, NSAIDs can be classified into three groups. These are 1) carboxylic acids, including salicylates (aspirin etc.), acetic acids (indomethacin etc.), propionic acid (CPF, etc.) and fenamates (flunixin etc.); 2) pyrazolones (PBZ etc.) and 3) oxicams (piroxicam etc.).

1.5.2.2. Side effects of NSAIDs

There are now more than 50 different NSAIDs on the human pharmaceutical market and there is a continuing flow of new preparations being introduced. About 10 NSAIDs are available in veterinary practice. The fact that so many new compounds have been produced and are still being produced is a reflection of the fact that none is ideal in controlling or modifying the signs and symptoms of inflammation, particularly in the common inflammatory joint diseases. A particular problem is that virtually all NSAIDs can have significant unwanted effects.

The main side effects of NSAIDs involve gastro-intestinal irritation and ulceration. Lesions may occur throughout the gastro-intestinal tract and may lead to a life-threatening plasma protein-losing enteropathy in the horse. These side effects are thought to be caused by the inhibition of COX-1 and thus inhibition of the formation of PGs, especially PGI_2 and PGE_2. These gastro-intestinal PGs serve as cytoprotective agents in the gastric mucosa. They promote perfusion of gastric and intestinal mucosa and mucus production and have an inhibitory influence on acid secretion (Frey, 1992). In addition, p.o. administered drugs produce local irritation which allows back diffusion of acid into the gastric mucosa and induces tissue damage.

Other side effects include vomiting, blood dyscrasias, hepatotoxicity due to cholestatic and parenchymal cell damage, thrombocytopaenia, leukopaenia, renal papillary necrosis and occasionally skin rashes.

It is believed that NSAIDs produce their antiinflammatory effects by inhibiting COX-2 and their side effects by inhibiting COX-1. It is, therefore, desirable that a NSAID should have good inhibitory effects for COX-2 with less inhibition for COX-1 in the target animal species. Most currently used NSAIDs are non-selective inhibitors of these two isoenzymes. This indicates that they produce therapeutic and side effects simultaneously. Selective COX-2 inhibitor of NSAIDs are now under development (Vane, 1994; Vane & Botting, 1995). It is anticipated that they produce therapeutic effects with less or no COX-1-associated side effects.

1.5.3. Investigation of the use of NSAIDs in sheep, goats and donkeys

Although no NSAIDs have been licensed for use in sheep, goats and donkeys, it is of clinical and scientific interests to investigate the potential uses of NSAIDs in these species since inflammation is a common pathophysiological process in these animals and undoubtedly the use of NSAIDs will improve the welfare of these animal species.

The present studies were set up to investigate the potential uses of some NSAIDs in sheep [FM, PBZ and CFP (including its enantiomers)], donkeys (FM and PBZ), goats (PBZ and PRT) and camels (PRT). In the studies in sheep and donkeys, a subcutaneous tissue-cage model of inflammation was used. The effects of the drugs on COX-2 was estimated by measurement of PGE_2 in carrageenan-induced inflammatory exudate, and exudate LTB_4 was quantified to assess the inhibitory effects of NSAIDs on 5-LOX. The effect of the drugs on COX-1 was determined by measuring TXB_2 generation by PLT COX during blood clotting. Changes in skin temperature over cages were used as a cardinal sign of acute inflammation. The disposition and distribution of the drugs in plasma, exudate and transudate were determined to provide the evidence for dosing and withdrawal schedules of the drugs. By application of PK/PD modelling, the relationships between drug effects and drug concentrations in plasma and therapeutic sites were established and parameters such as IC_{50} were used to quantitatively estimate and potency of therapeutic effects. In addition, the changes in blood leukocyte and PLT numbers were measured to estimate the effects of the drugs on the haematological system and the effects of the drugs on exudate leukocytes were determined to asses the therapeutic effects on leukocyte accumulation.

Another purpose of these studies was to elucidate inflammatory mechanisms in the target animals using NSAIDs as a tool. Reduced heat generated at the site of inflammation and parallel inhibition of eicosanoid synthesis by COX isoenzymes provided an indication of the possible role of eicosanoids in acute inflammation.

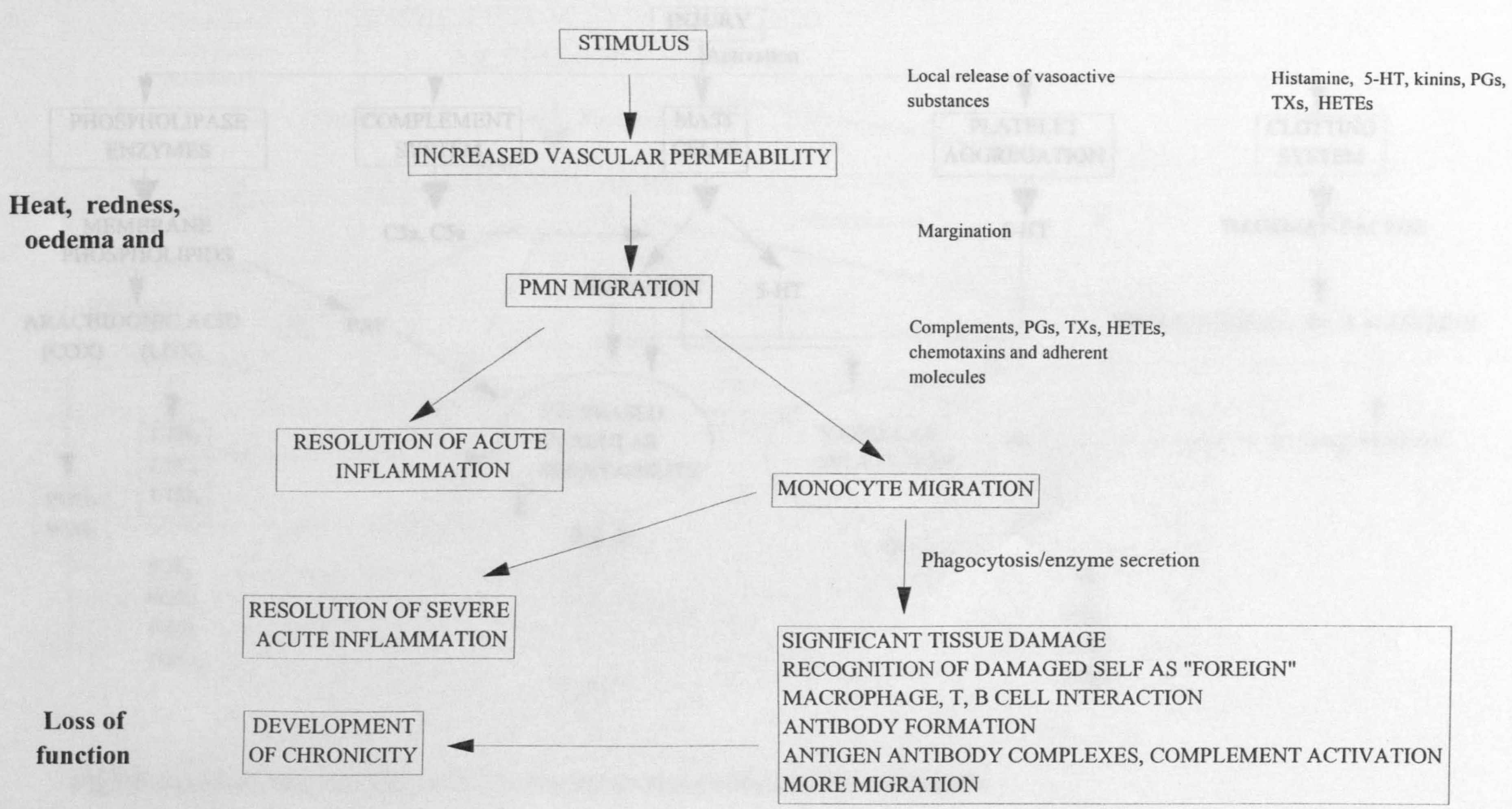


Fig. 1.1. Schematic representation of the inflammatory process.

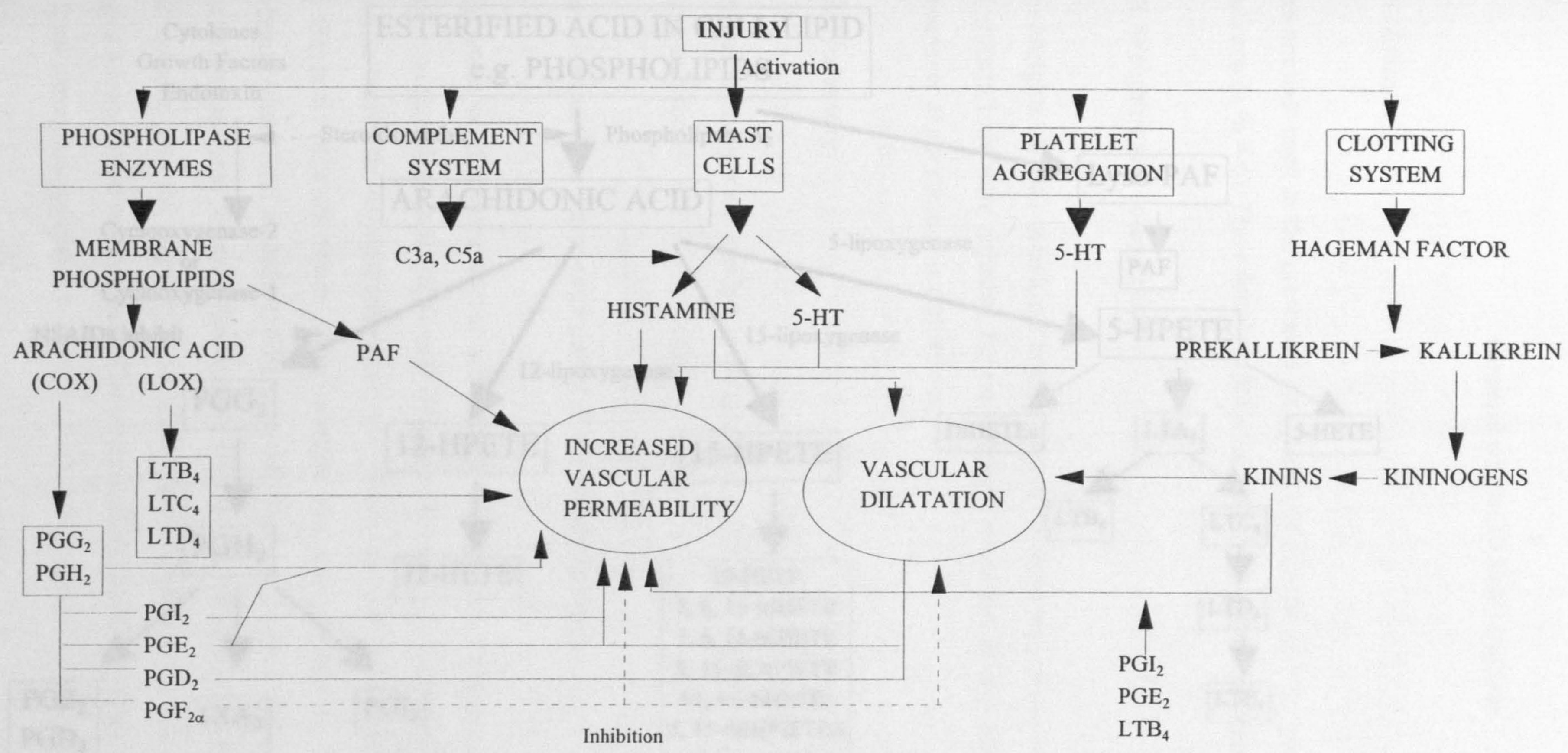


Fig 1.2. Schematic representation of inflammatory mediator effects on the vasculature.

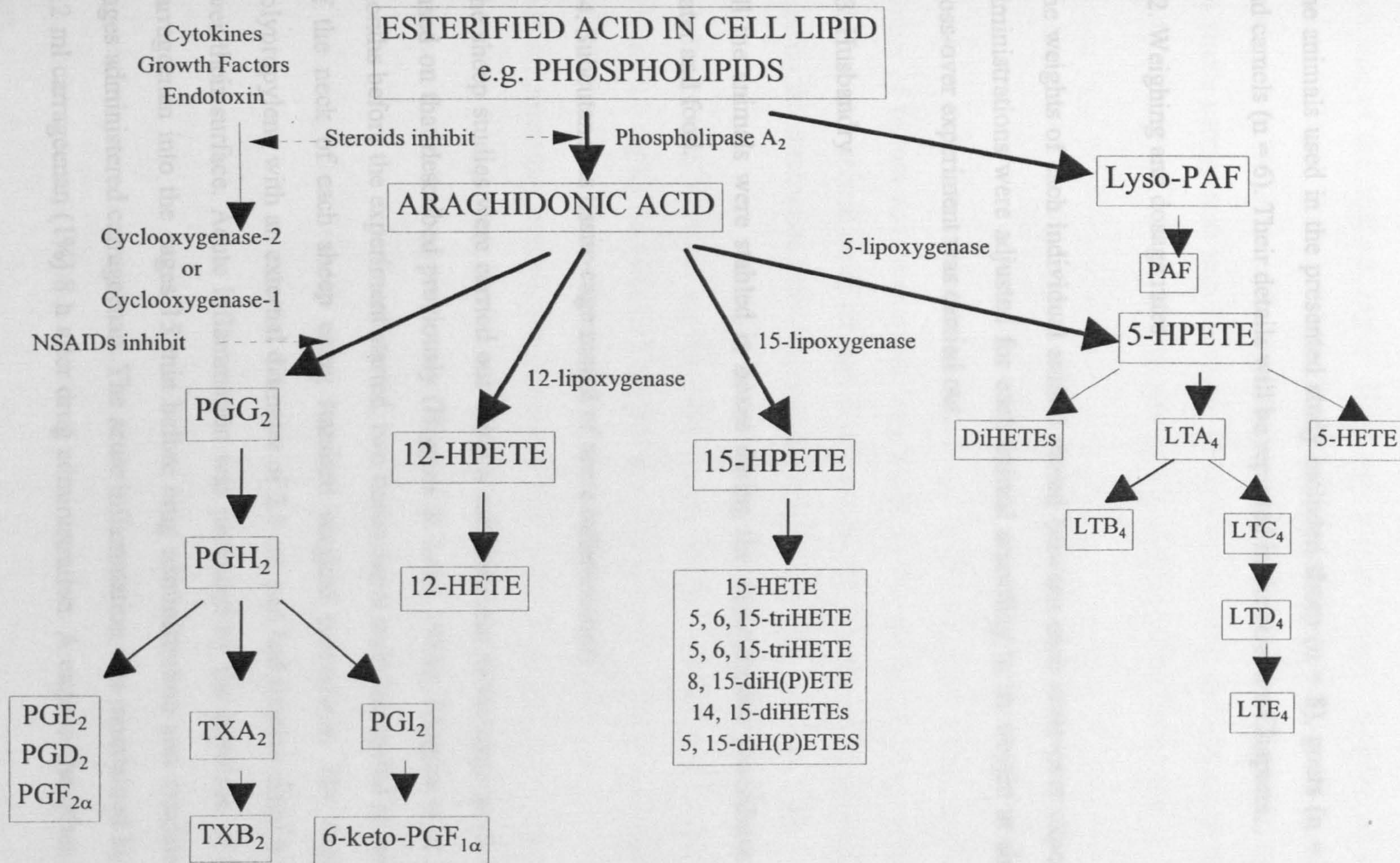


Fig 1.3. Metabolism of arachidonic acid.

Chapter 2

General materials and methods

2.1. Animals

The animals used in the presented study included sheep (n = 8), goats (n = 6), donkeys (n = 3) and camels (n = 6). Their details will be reported in the relevant Chapters.

2.2. Weighing and dosage rates

The weights of each individual animal varied between each cross-over experiment and the drug administrations were adjusted for each animal according to its weight at about 24 h before the cross-over experiment was carried out.

2.3. Husbandry

All the animals were stabled in boxes during the experimental procedures with free access to water and food.

2.4. Subcutaneous tissue-cage model of acute inflammation

The sheep studies were carried out using a subcutaneous tissue-cage acute inflammatory model based on that described previously (Higgins & Lees, 1984c; Higgins *et al.*, 1987). Briefly, two months before the experiment started, two tissue cages were implanted in the middle of each side of the neck of each sheep using standard surgical procedures. The cages were made from polypropylene with an external diameter of 2.0 cm and had 6 holes sized 0.5 cm in diameter cut over their surface. Acute inflammation was produced by the injection of 0.3 ml of 1% sterile carrageenan into the cages 15 min before drug administration and exudate was collected from cages administered carrageenan. The acute inflammation was maintained by a repeat injection of 0.2 ml carrageenan (1%) 8 h after drug administration. A cage in the other side of the neck into

which carrageenan was not injected was used for the collection of transudate. A different cage was used for carrageenan induction of inflammation on each the cross-over occasions.

The similar procedures were used for the development of a subcutaneous tissue-cage model of acute inflammation in donkeys. A multiperforated polypropylene practice golf ball was placed in each side of the midneck region. Each ball had an internal diameter of 41 mm and internal volume of 31 ml. Each ball had 25 holes, corresponding to about 9.3 % of total surface area, punched in it. The acute inflammatory process was induced by intracaveal injection of 0.5 ml of 1% sterile carrageenan into the cages 15 min before drug administration and maintained by a further injection of 0.5 ml 1% carrageenan 8 h following drug treatments.

2.5. Experimental protocol

The sheep study was carried out as a multiple factor cross-over using a Latin square design. The number of factors and cross-overs were equal to the number of drugs given plus PLB control such that each animal received each drug as well as PLB treatment. The wash-out period varied based on the elimination rate of drug in plasma in the target animal species.

The dosage rates were 1.1 mg/kg body weight for FM, 4.4 mg/kg for PBZ, 4.0 mg/kg for rac-CPF and 2.0 mg/kg for each CPF enantiomer. Placebo was physiological saline at a dose volume equivalent to the largest dose volume of the drugs. In the studies using subcutaneous tissue-cage acute inflammatory models, the drugs and PLB were given i.v. as a single dose and as a rapid bolus, into the right jugular vein according to the cross-over design, 15 min after the injection of carrageenan into the cage.

2.6. Sampling schedule

2.6.1. Temperature

Temperature was recorded using a directing-reading infra-red thermometer (Horiba, IT-330 infrared thermometer, Miyanohigashi, Kyoto, Japan) held approximately 1 cm above each tissue cage.

2.6.2. Plasma

Blood was collected in 10 ml Lithium Heparin Monovettes (Sarstedt Ltd, Numbrech, Germany). These were centrifuged at 1800 × g and 4 °C for 10 min and plasma decanted into polystyrene tubes. Tubes were stored at -20 °C until analysis of the drugs and their metabolites.

2.6.3. Serum

Approximately 3 ml of blood was collected in a plastic syringe and immediately transferred into a glass tube. The tubes were quickly transferred to a water bath at 37 °C for 90 min to allow clotting. The tube was then centrifuged at 1800 × g and 4 °C for 10 min. Supernatant (Serum) was then transferred into 2 ml plastic tubes and stored at -70 °C until analysis of TXB_2 concentration.

2.6.4. Blood

Blood for haematology was collected in plastic syringes and immediately transferred into 0.5 ml paediatric tubes containing K EDTA. Where possible, haematological determinations were carried out on the day of sampling.

2.6.5. Transudate

In order to prevent eicosanoidal generation *ex vivo*, tubes were prepared with the dual COX and LOX inhibitor, BW540C, as follows:

Fifty mg of BW540C was dissolved in 50 ml methanol. Ten microlitres of this solution was added to each sample tube and allowed to dry. Prior to use, 50 iu of heparin was also added to each tube.

Transudate was drawn into a plastic syringe and immediately placed in a tube containing BW540C and heparin. This was kept on an ice until centrifuged at 1800 × g and 4 °C for 10 min. Supernatant was collected and divided into two aliquots for determination of drugs and their metabolites.

2.6.6. Exudate

Exudate was collected into tubes prepared as for transudate. A 0.1 ml sample was taken prior to centrifugation for haematological determination. Following centrifugation (as for transudate) 0.1 ml aliquots of the remaining sample were pipetted into separate eppindorf tubes for PGE_2, 12-HETE and LTB_4 quantification, and a further sample taken as a spare. The remainder of the sample was retained for estimation of the drugs and their metabolites.

2.7. Drug analysis

2.7.1. Analysis of PBZ and OPBZ

2.7.1.1 Extraction and chromatography

The PBZ concentrations in plasma, exudate and transudate were determined by HPLC.

The standard concentrations of PBZ and OPBZ were prepared in methanol and serial dilutions of the initial stock solution were prepared at 1000, 500, 100, 20 and 5 µg/ml. To prepare the serial spikes, a range of standards containing PBZ of 0.00, 0.25, 1.00, 5.00, 25.00 and 100.00 µg/ml and OPBZ of 0.00, 0.10, 0.25, 1.00 and 5.00 µg/ml were made and then 1.0 ml blank plasma (or 0.2 ml of exudate/transudate) was added. For the sample analysis, a 1 ml sample of plasma (or a 0.2 ml of exudate/transudate) was pipetted into the appropriate tubes.

The spikes and samples were acidified with 0.2 ml (0.1 ml for exudate and transudate) citrate phosphate buffer (pH 3.0). After mixture using a vortex mixer, 6.0 ml (4 ml for exudate/transudate) chloroform was added and the contents mixed for 20 min using a slow rotary mixer. Following centrifugation at 1800 × g and 4 °C for 20 min, 4 ml (3 ml for

exudate/transudate) of the organic phase was removed and evaporated to 0.5 ml at 50 °C under a nitrogen stream and tubes were washed down with 0.5 ml of chloroform and further evaporated to dryness. The residue was reconstituted in 0.5 ml (0.2 ml for exudate/transudate) methanol and fully dissolved by vortexing for 10 seconds (sec), sonicating for 1 min and again vortexing for 10 sec.

The chromatography was carried out on a Spectraphysics Automated HPLC system which contains a computer (IBM, OS/2) data handling system with PC1000 chromatography software, a gradient pump, an autosampler and a variable UV detector. A C_{18} ODS Aiphasil 5 column (25 cm × 4.6 mm) plus a guard column was used. The mobile phase was water and acetonitrile containing 0.0007% perchloric acid and degassed with He gas. The gradient began with 50 % of acetonitrile and 50 % of water and ended at 80 % of acetonitrile and 20 % of water at 11 min. And then the starting gradient (80 % acetonitrile : 20 % water) was restored from 11 to 13 min. The injection volume was 20 µl and the running time was 13 min at a flow rate of 1.5 ml/min. The detector was focused on COM_2 at single wavelength mode at 254 nm. The results were calculated using the values of peak area.

2.7.1.2. Sensitivity and reproducibility of the assay

Phenylbutazone and OPBZ were well separated by this assay and the retention time was 4.5 min for OPBZ and 7.5 min for PBZ. The limit of quantification was 0.05 µg/ml. The percentage recovery (mean ± SE) was 97.93 ± 1.97 % for PBZ (n=20) and 96.11 ± 2.42 % for OPBZ (n=20). Reported values were corrected for extraction losses.

To evaluate the reproducibility of the analysis, the intra and inter assays of the spikes were carried out. The mean percentage recovery and Co-Var were calculated as the criteria for assessment. The results are given in Table 2.1. and Table 2.2.

To test the linearity of the assay the standard concentrations and spike concentrations were plotted versus the values of the peak area. A good linearity (r^2>0.99) was obtained.

2.7.2. Analysis of FM

The FM concentrations in plasma, exudate and transudate were determined by HPLC using the same extraction procedures and HPLC system and column as described above for PBZ. The mobile phase was 40 : 40 : 20 acetonitrile : methanol : water containing 0.04% glacial acetic acid at a flow rate of 1.5 ml/min and the wavelength was 287 nm. The limit of quantification was 0.05 μg/ml with a good linearity (r^2>0.99) for spike concentrations ranging from 0.1 to 25 μg/ml. The percentage recovery was 97.46±1.78 % (n=20, mean±SE). Reported values have been corrected for extraction losses. The details for sensitivity and reproducibility were given in Table 2.3.

2.8. Quantification of TXB_2

2.8.1. Radioimmunoassay procedures

Serum TXB_2 concentrations were determined using RIA methods previously validated by Higgins & Lees (1984a). Samples were analysed without prior extraction but diluted with buffer. The RIA was carried out as follows.

Buffer preparation: Gelatin (1.00 g) was dissolved in 800 ml water, and 0.1 g sodium azide, 6.61 g tris hydrochloride and 0.97 g tris base were added. This was made up to 1 L with water. The pH was adjusted to 7.4 by adding 1 M HCl or NaOH. Warming was required to dissolve the gelatin.

Dextran-coated charcoal: Dextran (0.40 g; T-70, Pharmacia Biotech Ltd, St. Albans, Herts, England) was dissolved in 80 ml water and stirred for approximately 1 h, 2.0 g charcoal (neutralised and activated, Sigma Ltd, Poole, Dorset, England) was added and stirred for 0.5 h. Water was added and made up to 100 ml. Following storage, it was thoroughly stirred before use. The dextran coated charcoal suspension was kept at 0-4 °C for a week at most before use.

Reconstitution and dilution of anti-TXB_2 serum: Stock powder (100 Tests/vial) was dissolved in 10 ml of buffer. This stock solution was stored at -20 °C. When used, it was diluted 10 times with buffer. The diluted antiserum was a working solution and was stored at 0-5 °C prior to use.

The antiserum was tested using standard curve where the ratio of zero standard counts (B_0) to total counts (TC) was in the range of 40-60 %. Appropriate sample dilutions was made such that the percentage of tracer binding (B/B_0) of the sample (pre-treatment sample or PLB-treated sample) was located in the middle of the analytic range of the standard curve.

Standards: The stock standard solution (1 mg/ml) of TXB_2 was prepared in absolute ethanol. A portion of it was further diluted in buffer to obtain standard solutions at 10, 5, 2.5, 1.25, 0.625, 0.32, 0.16 and 0.08 ng/ml.

Tracer: The tracer was (5, 6, 8, 9, 11, 12, 14, 15 (n) ^{3}H TXB_2 (25µci/vial). This was provided with the stock solution and was stored in the supervised area at -20 °C. The working solution was prepared by adding 6 µl of tracer to 10 ml buffer.

Radioimmunoassay protocol: The tubes were numbered in duplicate, and the assay was performed as follows:

Reagents	NSB	B_0	STDs	TC	Tests
Standard (µl)	-	-	100	-	-
Sample (µl)	-	-	-	-	100
Buffer (µl)	300	200	100	500	100
Antiserum (µl)	-	100	100	-	100
Tracer (µl)	100	100	100	100	100

NSB: Buffer blank. B_0: Zero standard. STD: Standard and TC: Total count.

All the tubes were vortexed and incubated at 4 °C for 16-24 h.

Charcoal/dextran (200 µl) was added to all tubes except total counts and they were vortexed before being incubated at -4 °C for 10 min. The tubes were centrifuged at 1800 × g for 10 min. The supernatant was decanted to 6 ml scintillation vials containing 4 ml scintillant. The vials were counted as cpm.

Calculation: The average cpm for each set of replicated tubes was used and $\%B/B_0$ was calculated using equation

$$\% B/B_0 = \frac{STD(sample)cpm - NSBcpm}{B_0 cpm - NSBcpm} \times 100$$

Standard curves were generated by plotting $\%B/B_0$ as a function of the $\log_{10}$ TXB_2 concentration and by linear regression, fitted to the equation

$$\text{Log } TXB_2 \text{ concentration} = b \times \%B/B_0 + a$$

where 'b' was ratio and 'a' was intercept. Replacement of STD % B/B_0 with sample $\%B/B_0$ generated the values of TXB_2 concentration in the samples. These calculation procedures were carried out using the computer programme in Microsoft Excel 5.0 via a function of "GROWTH":

$$\text{Sample } TXB_2 \text{ concentration} = \text{GROWTH}(TXB_2 \text{ STDs}, \%B/B_0 \text{ STDs}, \text{sample } \%B/B_0)$$

2.8.2. Characterisation of the assay

In order to asses the linearity, available analytic range and reproducibility of the RIA, a plot of $\%B/B_0$ versus logarithmic TXB_2 concentrations was prepared (Fig. 2.1.). This indicates that the available range of the assay was 0.08-5.0 ng/ml. The $\%B/B_0$ range was 93.91 ± 2.96-1.54 ± 0.95 %. The concentration of TXB_2 which produces 50% of $\%B/B_0$ (BC_{50}) was 0.34 ± 0.01 ng/ml. The linearity (r^2) was 0.96 ± 0.01 (mean ± SD, n=9) where the regressive relationship (b) was statistically significant ($P<0.01$). The limit of quantification was 0.08 ng/ml in the standard curve so that the actual limit of quantification for the sample was 0.08 ng/ml × dilution times.

2.9. Quantification of PGE_2

Concentrations of PGE_2 in exudate were determined by RIA using the same procedures as described for TXB_2 but using PGE_2 standard, PGE_2 tracer and anti-PGE_2 serum.

The standard curves of 6 assays show that the available analytic range of the assay was 0.08 - 10.0 ng/ml of PGE_2, the $\%B/B_0$ range was 99.82 ± 1.46 - 3.93 ± 1.90 %, the BC_{50} was 1.12 ±

0.04 ng/ml of PGE_2 standard and the r^2 was 0.98 ± 0.01 and regressive relationship (b) was significant (P<0.01). (Fig. 2.3.).

2.10. Quantification of LTB_4

2.10.1. Radioimmunoassay procedures

Concentrations of LTB_4 in exudate were measured by RIA using LTB_4 standard, LTB_4 tracer and anti-LTB_4 serum. The procedures described for the TXB_2 RIA were used but with some modifications. Before LTB_4 tracer was added, the vials were incubated at 4 °C for 30 min. After dextran-coated charcoal was added, incubated and centrifuged, 0.3 ml of the supernatant was removed into the 6 ml scintillation vials containing 3 ml scintillant. The vials were counted as cpm.

2.10.2. Characterisation of the assay

To evaluate the characteristics of the assay, 4 assays for standard curves were carried out. The plot of $\%B/B_0$ versus logarithmic LTB_4 standard concentrations is given in Fig. 2.3. This indicates similar characteristics of the assay to the RIA for TXB_2. The linearity was 0.92 ± 0.001 and the regressive relationship (b) was significant (P<0.01). The maximal $\%B/B_0$ was 96.12 ± 1.44 % and the minimal $\%B/B_0$ was 2.31 ± 0.78 %. The BC_{50} was 0.47 ± 0.01 ng/ml. The available analytic range was 0.08-5.0 ng/ml of LTB_4.

2.11. Haematological determinations

Haematological variables determined on blood and exudate samples included leukocyte (including classification), erythrocyte numbers, haemoglobin concentration, haematocrit and PLT count. All values were determined using a Roche, Cobas, Minos, Vet. Automated Haematology Analyser (Roche Products LTD, Hertfordshire, England).

2.12. Pharmacokinetic analysis

2.12.1. Compartmental modelling

Compartmental parameters were estimated by non-linear least square regression using PCNONLIN 4.0 software programme (Scientific Consulting Inc., North Carolina, USA) with Nelder Mead algorithm. The initial estimates were calculated by the software using the curve stripping method of residuals (Gibaldi & Perrier, 1982a) based on the model fitted.

Two compartmental and three-compartmental models with bolus input and first order output were used for the data of plasma drug concentration versus time during model selection, thus,

$$C(t) = Ae^{-\alpha t} + Be^{-\beta t}.$$

$$C(t) = Ae^{-\alpha t} + Pe^{-\pi t} + Be^{-\beta t}.$$

Where A, P, and B are intercept terms and α, π and β are rate constants of fast distribution, slow distribution and elimination, respectively. The decision for the best fitting model was made by MAICE based on the AIC (Yamaoka *et. al.*, 1978). The AIC values were calculated from the non-linear regression results. In order to strengthen the fitting in the elimination phase of the curve, a weighting factor (1 over the drug concentrations) was used for the non-linear regression.

Area under the curve and AUMC were calculated by:

$$AUC_{0-\infty} = \frac{A}{\alpha} + \frac{B}{\beta}$$

and

$$AUMC_{0-\infty} = \frac{A}{\alpha^2} + \frac{B}{\beta^2}$$

for the two compartmental model, or

$$AUC_{0-\infty} = \frac{A}{\alpha} + \frac{P}{\pi} + \frac{B}{\beta}$$

and

$$AUMC_{0-\infty} = \frac{A}{\alpha^2} + \frac{P}{\pi^2} + \frac{B}{\beta^2}$$

for the 3 compartment model. And the MRT was calculated by

$$MRT_{0-\infty} = \frac{AUMC}{AUC}$$

Plasma distribution half-lives ($t_{\frac{1}{2}\alpha}$ and $t_{\frac{1}{2}\pi}$) and the elimination half-life ($t_{\frac{1}{2}\beta}$) were calculated as 0.693/α, 0.693/π and 0.693/β, respectively. The Vc was calculated as dose divided by C_p^0 which is calculated by summing the intercepts of the exponential equations. Body clearance was calculated as dose divided by AUC. Volume of distribution at steady state was calculated as MRT $\times$ Cl_B.

The data for exudate (transudate) drug concentration - time were fitted to a one compartment model with first-order input, first-order output and a lag time.

$$C(t) = M(e^{-k10(t-tlag)} - e^{-k01(t-tlag)})$$

where t_{lag} is lag time, M is the hybrid parameter, k_{01} is the absorption rate (distribution from plasma into tissue-cage fluids) and k_{10} is the elimination rate. Absorption half life was calculated as 0.693/k_{01}, $t_{\frac{1}{2}k10}$ was calculated as 0.693/k_{10} and AUC was calculated as

$$AUC_{0-\infty} = M(\frac{1}{k_{10}} - \frac{1}{k_{01}})$$

2.12.2. Non-compartmental modelling

The drug concentration time data from plasma, exudate and transudate were also analysed by non-compartment modelling using PCNONLIN 4.0 software programme.

The AUC was estimated using a linear trapezoidal equation

$$AUC_{0-last} = \sum_{i=1}^{n} \frac{C_i + C_{i-1}}{2} \times (t_i - t_{i-1})$$

and

$$AUC_{0-\infty} = AUC_{0-last} + \frac{C_{last}}{\beta}$$

where C represents the plasma concentration, i-1, and i are adjacent data point times, C_{last} denotes the concentration at the last sampling time or the last detectable concentration. β was first order rate constant associated with the terminal (log-linear) portion of the curve and was estimated using the algorithms as described by Dunne (1985). The AUMC was calculated using the equation

$$AUMC_{0-last} = \sum_{i=2}^{n} \frac{C_i t_i + C_{i-1} t_{i-1}}{2} \times (t_i - t_{i-1}).$$

The extrapolation to infinity was calculated using

$$AUMC_{last-\infty} = \frac{t_{last} \times C_{last}}{\beta} + \frac{C_{last}}{\beta^2}$$

and

$$AUMC_{0-\infty} = AUMC_{0-last} + AUMC_{last-\infty}$$

Thus MRT was calculated as

$$MRT_{0-last} = \frac{AUMC_{0-last}}{AUC_{0-last}}$$

and

$$MRT_{0-\infty} = \frac{AUMC_{0-\infty}}{AUC_{0-\infty}}$$

and MAT was calculated as:

$$MAT_{0\text{-last}} = MRT_{0\text{-last p.o. (or tissue cage fluids)}} - MRT_{0\text{-last i.v.(plasma)}}.$$

and

$$MAT_{0-\infty} = MRT_{0-\infty \text{ p.o. (or tissue cage fluids)}} - MRT_{0-\infty \text{ i.v.(plasma)}}.$$

Body clearance was calculated as dose/AUC and V_{ss} was calculated as MRT multiplied by Cl_B.

2.12.3. Bioavailability and distribution rate into tissue cage fluids

Bioavailability was determined using the equation

$$F = \frac{AUC_{oral} \times Dose_{iv}}{AUC_{i.v.} \times Dose_{oral}} \times 100$$

In order to correct for the differences in $t_{\frac{1}{2}\beta}$ following each route of administration, the above equation was corrected as:

$$F = \frac{AUC_{oral} \times Dose_{iv} \times t_{\frac{1}{2}\beta i.v.}}{AUC_{i.v.} \times Dose_{oral} \times t_{\frac{1}{2}\beta oral}} \times 100$$

The extent of drug distribution into tissue-cage fluids was calculated by:

$$\frac{AUC_{cage}}{AUC_{plasma}} \times 100\%$$

where AUC_{cage} is the area under exudate or transudate drug concentration time curve and AUC_{plasma} is the area under plasma drug concentration time curve. In consideration of the differences of the elimination rates between plasma and tissue-cage fluids, which affects the values of AUC, a correction was made by:

$$\frac{AUC_{cage} \times MRT_{plasma}}{AUC_{plasma} \times MRT_{cage}} \times 100\%$$

where MRT_{plasma} is the mean residence time of the drug in plasma and MRT_{cage} is the MRT of the drug in exudate or transudate.

2.12.4. Units of PK parameters

The units of dose, drug concentration and time used for PK modelling were μg/kg body weight, μg/ml and h, respectively. Accordingly the units of the parameters were generated as: A, P, and B (μg/ml), α, π and β (h^{-1}), C_p^0 (μg/ml), $t_{\frac{1}{2}\alpha}$, $t_{\frac{1}{2}\pi}$ and $t_{\frac{1}{2}\beta}$ (h), MRT (h), AUC (μg.h/ml), Vc (ml/kg), V_{ss} (ml/kg) and Cl_B (ml/kg/h).

2.13. Pharmacodynamic analysis

Pharmacodynamic analysis was carried out using PCNONLIN. The data of drug effect versus time were fitted to a Sigmoid inhibitory effect model

$$E = E_{max} - \frac{E_{max} \times T^{\gamma}}{T^{\gamma} + ET_{50}^{\gamma}}$$

and the data of effect versus drug concentration were fitted to a Sigmoid E_{max} model

$$E = \frac{E_{max} \times C^{\gamma}}{C^{\gamma} + EC_{50}^{\gamma}}$$

where E is the drug effect expressed in percentage inhibition, E_{max} is the maximal inhibitory effect, γ is the Hill constant, T is the time, ET_{50} is the time at which the drug effect declines by 50 % of the E_{max}, EC_{50} (IC_{50}) is the drug concentration producing 50% of the E_{max} , and C is the drug concentration in plasma for the estimation of serum TXB_2 inhibition or in exudate for the estimation of exudate PGE_2 inhibition.

Percentage inhibition of TXB_2 generation was calculated using the equation

$$\text{Inhibition (\%)} = (1 - \frac{TXB_i}{TXB_0}) \times 100$$

where TXB_i were the TXB_2 concentrations following drug administration at sequential sampling time i and TXB_0 was TXB_2 concentrations at 20 min prior to drug administration. Percentage inhibition of PGE_2 generation was calculated as:

$$\text{Inhibition (\%)} = (1 - \frac{PGE_{2(drug)}}{PGE_{2(PLB)}}) \times 100$$

where $PGE_{2(drug)}$ was exudate PGE_2 concentration following the drug administration and $PGE_{2(PLB)}$ was exudate PGE_2 concentration following PLB administration at the same time point in the same animal.

For some drugs with quick elimination rates (FM in the present study), the effects lasted for a long time after the drug became undetectable in the associated effect sites. In this case it was inapplicable to use the effect against drug concentration for the modelling purposes. The drug concentrations were simulated using corresponding PK equations (a two compartment model with bolus input and first-order output for the data of plasma drug concentration versus time and a one compartment model with first-order input and a lag time for the data of exudate drug concentration versus time). Thus: 1) compartmental modelling was carried out to obtain an equation for prediction of the drug concentration-time relationship in plasma and exudate, 2) the data of drug effect against time was fitted to a Sigmoid inhibitory effect model to obtain E_{max} and IT_{50} for serum TXB_2 and exudate PGE_2 generation and 3) IT_{50} was used to replace the independent variable (time) in the above PK equation to achieve the values of IC_{50}.

2.14. Statistical analysis

Most PD data in the Latin square design cross-over studies were analysed using ANOVA with repeated measurements. Balanced ANOVA was used for the experimental data without missing data. The GLM was used for experiments with missing data. These methods took account of factors including sequence, period, treatment and time as main effects and their associated two factor interactions. The analysis provided tests for differences in the factors and interactions.

Ideally a model for ANOVA should include the variances from all factors and interactions. However practically due to the limited numbers of experimental groups and their nested data, and a limit of the ranking, the ANOVA analysis could not fit sufficient factors in a single model. Most data were, therefore, analysed using the model:

$$Y = \mu + \text{treatment} + \text{time} + \text{animal} + \text{treatment} \times \text{time} + \text{treatment} \times \text{animal} + \text{time} \times \text{animal} + e$$

where μ is overall mean and e is residual error. This model provided tests for differences between treatment groups, time courses, animal individuals and their interactions. One of the important interactions was treatment × time, which provided the information for the differences of measurements collected at each time point between treatment groups. The effects of experimental period, sequence and their interactions were analysed using the model: $Y = \mu + \text{period} + \text{sequence} + \text{period} \times \text{sequence} + e$. In order to analyse effects of initial conditions (including the values of pre-treatment and normal concentrations of eicosanoids) and ambient temperature on the results, analysis of covariance was carried out by addition of those factors, as covariates, to the above model. All factors were treated as fixed effects except for animals which were a random factor.

The statistical analyses were undertaken with ANOVA using a MINITAB Software package (Version 10.1, Minitab Inc., State College, PA, USA). All tests for significance were carried out at a 0.05 level unless otherwise stated. Significant differences between treatment groups and between treatment × time interaction were further investigated using a Fisher's multiple comparison at an individual error rate of 0.05. The Fisher's intervals were calculated as:

$$\text{upper interval} = \bar{Y} + t \times s \times \sqrt{\frac{1}{n_1} + \frac{1}{n_2}}$$

and

$$\text{lower interval} = \bar{Y} - t \times s \times \sqrt{\frac{1}{n_1} + \frac{1}{n_2}}$$

where $\bar{Y}$ is the mean value, t is the Student's t distribution with a degree of freedom for error (0.05) from ANOVA, s is the pooled SD (obtained from ANOVA), and n_1 and n_2 are the number of observations in the levels compared. If the mean of the measurements fell within the Fisher's interval, it was considered that there was no statistical difference ($P>0.05$). Otherwise, it was statistically different ($P<0.05$).

2.15. Drugs and chemicals

2.15.1. Drugs for administration

The following drugs and chemicals were purchased: Phenylbutazone for i.v. administration (Phenyzone, C-Vet Ltd, Raintree, Essex, England), Phenylbutazone paste for p.o. administration (Equipalazone paste, Vet Drug Co., Hondon, England) and FM for i.v. administration (Finadyne, Schering-Plough Animal Health, Mildenhall, Suffolk, England).

Racemic CPF for i.v. administration were a gift from the Grampian Pharmaceutical Company (Talkin, Cumbria, Scotland). Excipient was also supplied by Grampian Pharmaceutical Company in order to formulate the enantiomers in solution.

2.15. Chemicals

The following chemicals were purchased: standards and antiserums of TXB_2, PGE_2 and LTB_4 (Sigma Ltd, Poole, Dorset, England); (5, 6, 8, 9, 11, 12, 14, 15 (n) ^3H-TXB_2, (5, 6, 8, 11, 12, 14,

15 (n) ^{3}H-PGE_2 and ^{3}H-LTB_4 (Amersham Ltd, Little Chalfont, Bucks, England); PBZ, OPBZ, rac-CPF standard, PRT, PRT glucuronide and L-NAME (Sigma Ltd, Poole, Dorset, England).

The following chemicals were gifted: BW540C (Wellcome Research Laboratories, Beckenham, Kent, England), FM standard (Schering-Plough Animal Health, Mildenhall, Suffolk, England), (-)(R)-CPF and (+)(S)-CPF (Professor P. Delatour, Ecole Nationale Veterinaire de Lyon, France) and PRT sulfate (McNeil Consumer Products, Fort Washington, PA, USA).

Table 2.1. Percentage recoveries and coefficients of variation (Co-Var) of PBZ in plasma spikes

Conc. (μg/ml)	Assay 1 (%)	Assay 2 (%)	Assay 3 (%)	Assay 4 (%)	Mean (%)	SD	SE	CO-Var (%)
0.00	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
0.25	85.74	81.95	104.92	89.21	90.46	10.09	5.04	11.15
1.00	90.83	102.45	90.68	95.20	94.79	5.52	2.76	5.83
5.00	94.31	99.96	91.81	101.04	96.78	4.44	2.22	4.58
25.00	88.18	109.10	102.04	99.53	99.71	8.69	4.35	8.72
100.00	101.83	115.26	103.80	110.79	107.92	6.22	3.11	5.76
Mean	92.18	101.74	98.65	99.16				
SD	6.26	12.57	6.85	7.96				
SE	2.80	5.62	3.06	3.56				
CO-Var	6.80	12.36	6.94	8.03				

Table 2.2. Percentage recoveries and coefficients of variation of OPBZ in plasma spikes

Conc. (mg/ml)	Assay 1 (%)	Assay 2 (%)	Assay 3 (%)	Assay 4 (%)	Mean (%)	SD	SE	CO-Var (%)
0.00	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
0.10	79.23	110.19	110.95	91.69	98.01	15.36	7.68	15.67
0.25	88.84	85.38	104.96	89.20	92.10	8.75	4.37	9.50
0.50	89.40	99.63	86.89	95.75	92.92	5.82	2.91	6.27
1.00	92.61	77.21	109.55	88.74	92.03	13.39	6.69	14.55
5.00	110.52	100.20	110.78	100.50	105.50	5.95	2.98	5.64
Mean	92.12	94.52	104.63	93.18				
SD	11.44	13.11	10.21	4.95				
SE	5.11	5.86	4.56	2.21				
CO-Var	12.41	13.87	9.75	5.31				

Table 2.3. Percentage recoveries and coefficients of variation of FM in plasma spikes

Conc. (μg/ml)	Assay 1 (%)	Assay 2 (%)	Assay 3 (%)	Assay 4 (%)	Mean (%)	SD (%)	SE (%)	Co-var (%)
0.00	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
0.25	95.56	81.44	91.02	87.20	88.81	5.98	2.99	6.74
1.00	96.88	91.62	103.97	90.24	95.68	6.22	3.11	6.51
5.00	101.22	98.94	100.05	97.64	99.46	1.53	0.77	1.54
25.00	111.50	109.98	103.88	98.30	105.92	6.05	3.03	5.71
Mean	101.29	95.50	99.73	93.35				
SD	7.22	12.03	6.09	5.49				
SE	3.61	6.02	3.04	2.74				
Co-var	7.13	12.60	6.10	5.88				

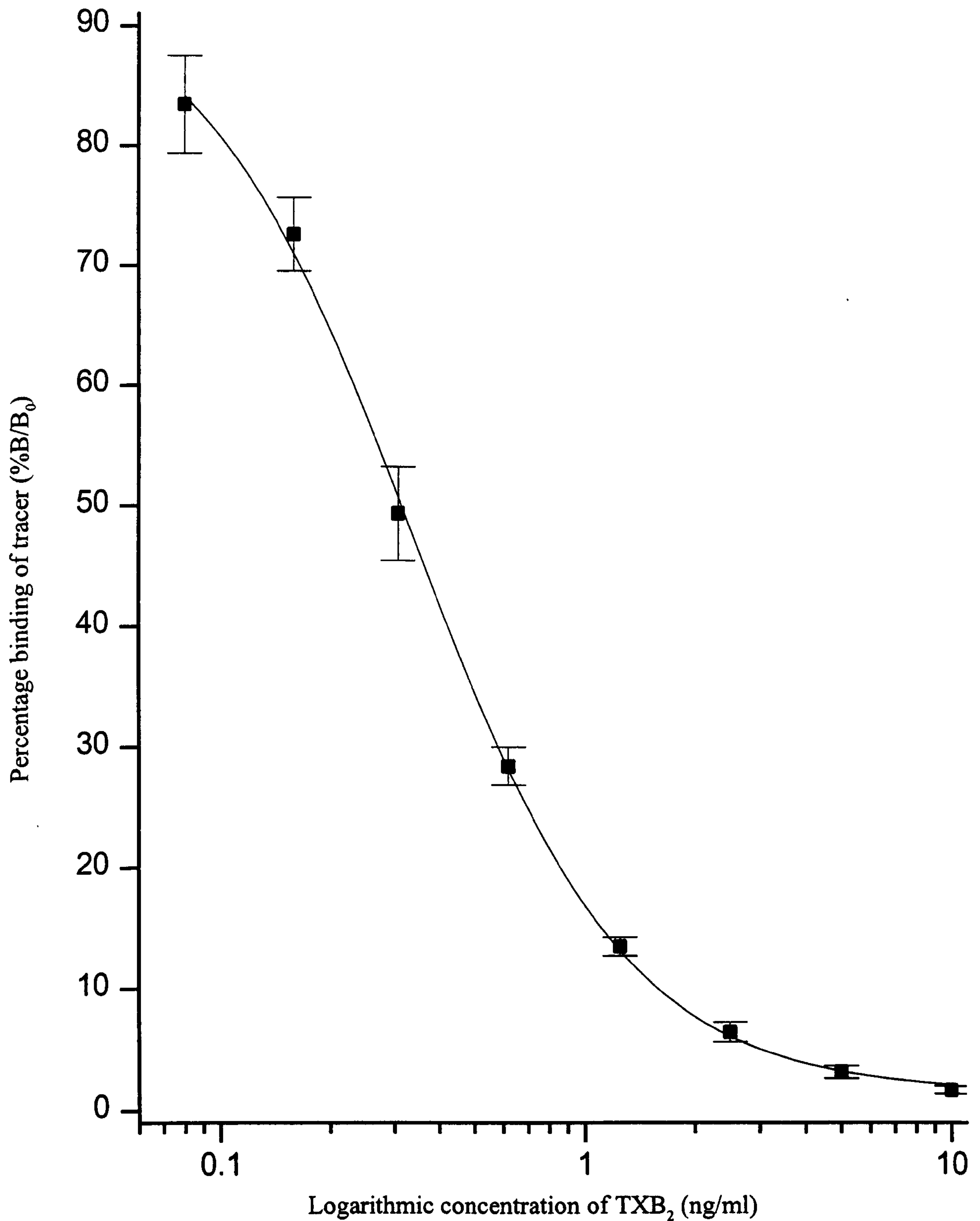


Fig. 2.1. Standard curve for percentage tracer binding (%B/B_0) versus TXB_2 concentrations to evaluate the linearity and reproducibility of the RIA (mean ± SD, n=9)

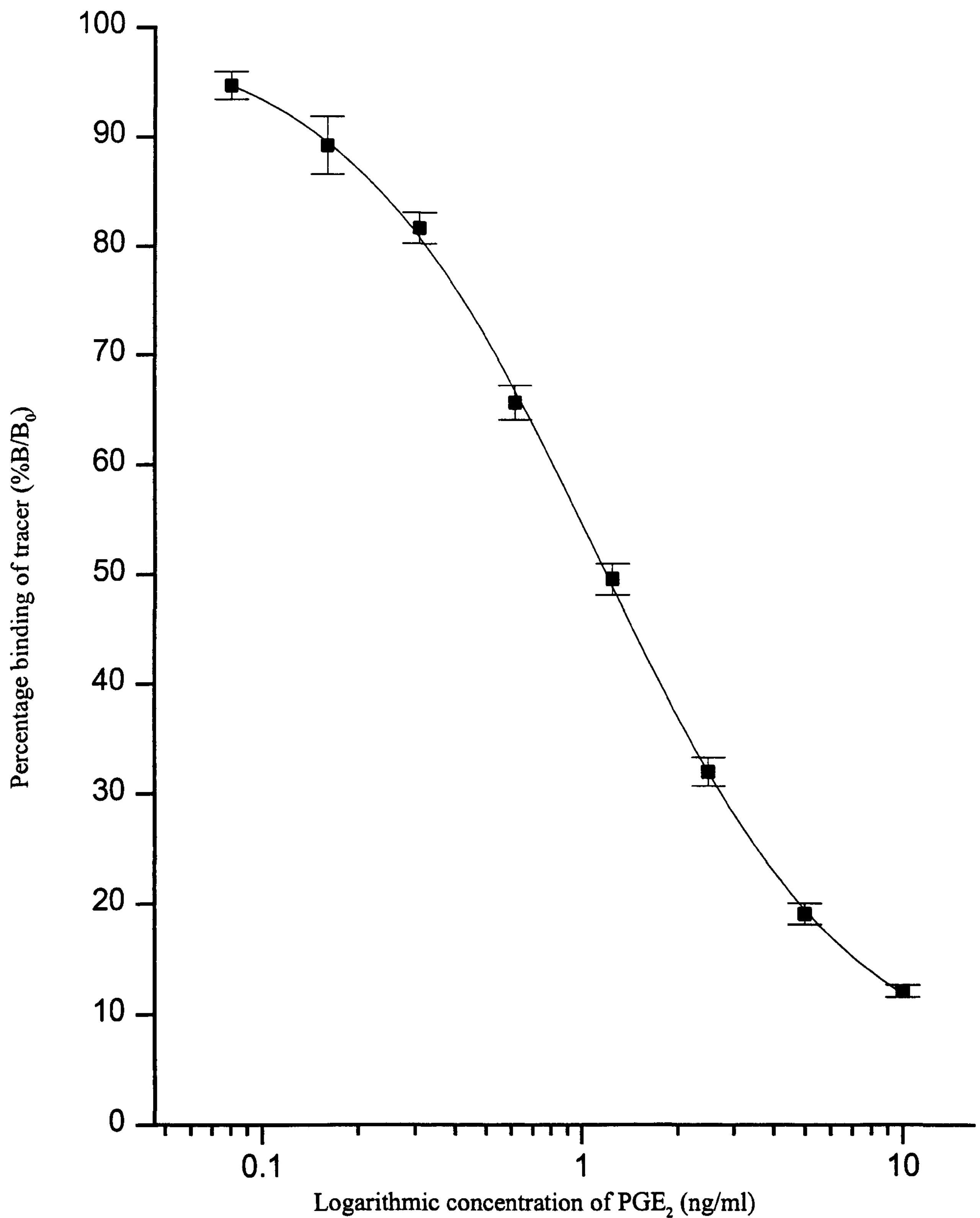


Fig. 2.2. Standard curve for percentage tracer binding (%B/B_0) versus PGE_2 concentrations to evaluate the linearity and reproducibility of the RIA (mean ± SE, n=6).

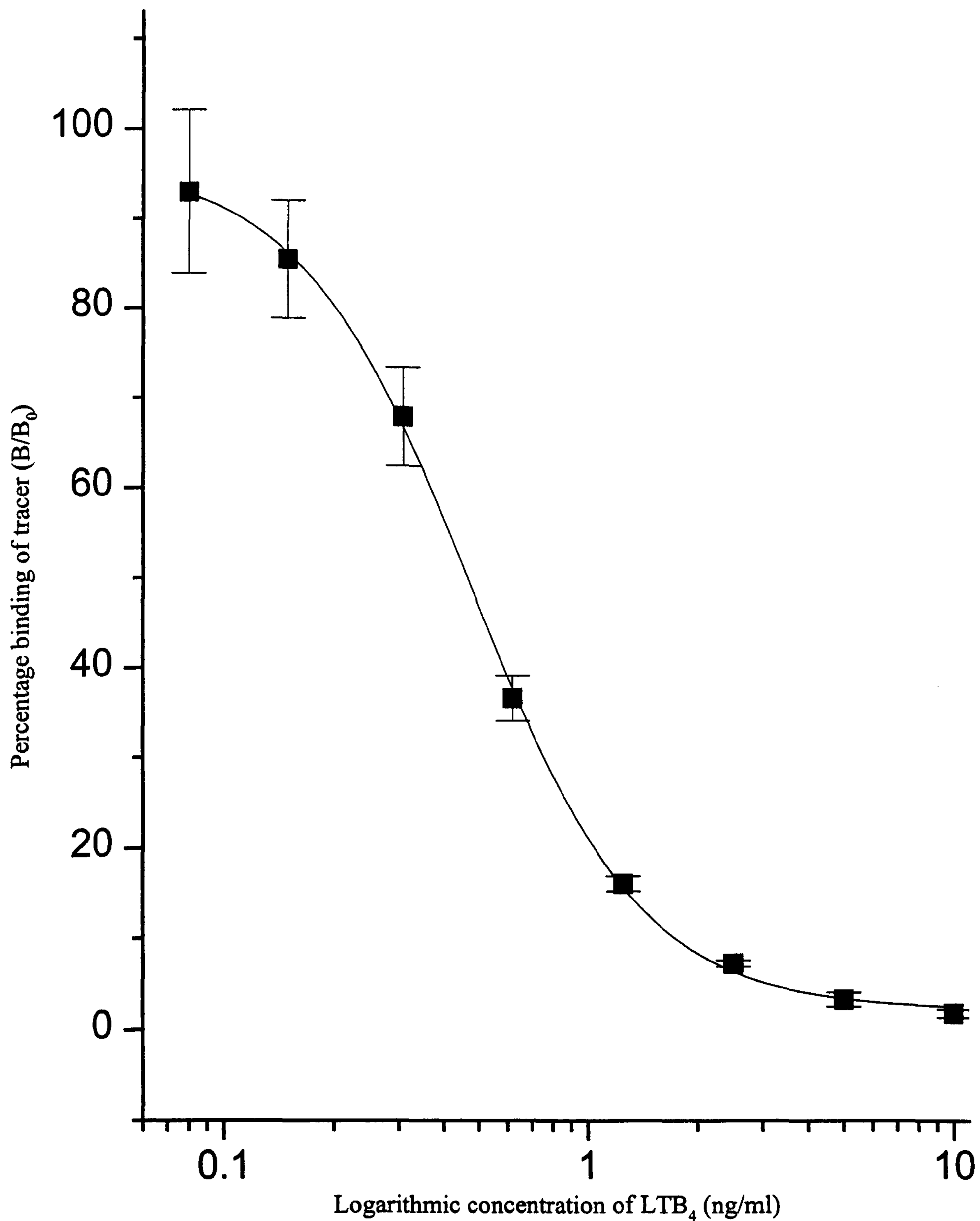


Fig. 2.3. The standard curve of percentage tracer binding versus logarithmic LTB_4 to evalute the sensitivity linearity and reproducibility of the RIA (n=4, mean ± SE).

Chapter 3

Pharmacokinetic and pharmacodynamic studies of flunixin meglumine and phenylbutazone in sheep

3.1. Introduction

Phenylbutazone (Fig. 3.1.) and FM (Fig. 3.1.) are NSAIDs classified as enolic and carboxylic acids, respectively. In common with other NSAIDs, they produce their principal effects by inhibiting COX enzymes and thus, blocking the formation of COX derived eicosanoid inflammatory mediators, such as PGs which are fundamental to the acute inflammatory process (Vane, 1971). PBZ and FM have been used widely for their antiinflammatory and analgesic properties to treat musculoskeletal conditions and colic in equine practice (Lees & Higgins, 1985; Tobin *et al.*, 1986). They are also used routinely in ruminant veterinary practice, in the treatment of acute mastitis, endotoxaemia and pneumonia in cattle and goats (Anderson *et al.*, 1986, 1991; Lohuis *et al.*, 1989), and FM was also used to treat endotoxin-induced reticulorumen stasis and tachycardia in cattle (Eades, 1993). Studies in our laboratory in carrageenan-induced hyperalgesia have demonstrated that FM is a potent analgesic in sheep (Welsh & Nolan 1994a, 1994b). The PK of PBZ have not been described in sheep although the plasma kinetics of FM have been investigated previously (Welsh *et al.*, 1993). For most NSAIDs their antiinflammatory effects last longer than would be expected from the plasma drug disposition. Previous studies in horses (Lees *et al.*, 1986; Lees *et al.*, 1987c) and calves (Landoni *et al.*, 1995a) showed that FM and PBZ have greater penetration into inflamed tissue fluid (exudate) than into non-inflamed transudate and are eliminated more slowly from the exudate than from plasma. These findings indicate that the relationships between plasma concentrations and effects are complex and that tissue kinetics in exudate may provide useful information.

Sheep COX-1 and COX-2 isoenzymes have been identified and purified and are used as a rich natural source of COX-1 and COX-2 for inflammatory studies (Ouderaa *et al.*, 1977; Johnson *et al.*, 1995). Previous studies *in vitro* using sheep COX-1 and COX-2 illustrated that these two isoenzymes had distinguished sensitivity to NSAIDs (Smith & Marnett, 1991; Johnson *et al.*,

1995). However *in vivo* studies on COX isoenzymes and their inhibition by NSAIDs in sheep have not been carried out.

The objectives of the present study were to i) establish the disposition of PBZ, OPBZ and FM in plasma, inflammatory tissue-cage fluids (exudate) and non-inflammatory tissue-cage fluids (transudate); ii) investigate the effects of PBZ and FM on COX and LOX enzymes. The inhibition of COX by the NSAIDs was estimated by determination of serum TXB_2 concentration generated by PLT in clotting blood and of exudate PGE_2 concentration generated by carrageenan-induced inflammation in the subcutaneous tissue-cage. Leukotriene B_4 in exudate was measured to estimate the effects of the drugs on 5-LOX; iii) investigate the effects of the NSAIDs on the recruitment of inflammatory cells in exudate and iv) establish the relationship between the drug concentrations and effects using PK/PD modelling.

3.2. Materials and methods

3.2.1. Animals

Eight male sheep weighing 45 ± 5 kg (mean ± SD) and aged approximately 1 year old at the beginning of the first cross-over were used. Hay and water were provided *ad libitum*.

3.3.2. Experimental protocol

The study was carried out using a subcutaneous tissue-cage acute inflammatory model as described in Chapter 2. A three way cross-over Latin square design was used for the study such that each sheep received PLB, FM and PBZ (Table 3.1.). A 21 day wash-out period was allowed between each cross-over. Fifteen min after the injection of carrageenan into the cage, FM at 1.1 mg/kg body weight, PBZ at 4.4 mg/kg body weight and PLB (normal saline) at a dose volume equivalent to FM were administered i.v., as a rapid bolus, into the right jugular vein according to the cross-over design.

Table 3.1. Cross-over design for i.v. administration of PBZ (A, 4.4 mg/kg), FM (B, 1.1 mg/kg) and PLB (C, saline) in 8 sheep

	Cross-over occasion		
Sheep No.	1	2	3
143	A	B	C
144	B	C	A
145	C	A	B
146	C	B	A
147	A	C	B
148	B	A	C
149	A	B	C
150	B	C	A

All of the injection preparations were made up to an equal volume of 5.0 ml by addition of 0.9 % physiological saline.

Blood samples were collected at -20 min (20 min before drug administration), 5, 15, 30, 45 min and 1, 2, 4, 6, 8, 12, 24, 32, 48, 72, 96, 120 and 144 h via the left jugular vein. Transudate and exudate samples (approximately 1 ml) were collected at -15 min (transudate only) and 2, 4, 8, 12, 24, 32, 48, 72, 96, 120 and 144 h. All of the samples were processed as described in Chapter 2.

The PBZ, OPBZ and FM concentrations in plasma, exudate and transudate was determined by HPLC as described in Chapter 2. The quantification of serum TXB_2, exudate PGE_2 and exudate LTB_4, and the haematological determinations were carried out according to the methods described in Chapter 2.

3.3.3 Pharmacokinetic and PD analysis

Pharmacokinetic and PD analysis were carried out following the methods described in Chapter 2. Compartmental and non-compartmental modelling were used for concentration time data in plasma, exudate and transudate. The IC_{50} of FM for serum TXB_2 and exudate PGE_2 were simulated by the PK equations.

3.3.4. Statistics

The results were expressed in mean ± SE. A three factor ANOVA (treatments of PBZ, FM and PLB) with multiple ways (treatments, sheep, times, periods and sequences) was carried out using a balanced design routine as described in Chapter 2. The effects of ambient temperature and pre-treatment values on the experimental parameters were estimated using an analysis of co-variance routine. Sheep were treated as random factors and other factors were treated as fixed. The differences in measurements collected at each time point between treatment groups, and between pre-treatment and post-treatment were further confirmed using Fisher's multiple comparisons following the ANOVA. The differences between individual animal, time points, cross-over sequences and cross-over periods and their two factor interactions were analysed using the ANOVA but without carrying out Fisher's multiple comparisons.

3.4. Results

3.4.1. Pharmacokinetics

3.4.1.1. Phenylbutazone

The PK data of PBZ and OPBZ in plasma, exudate and transudate are given in Tables 3.2.-3.6. and Figs. 3.2. and 3.3.

Following i.v. administration of PBZ at 4.4 mg/kg in sheep, the elimination of drug concentration from plasma was very slow (Fig. 3.2.). At 144 h post-administration PBZ was still detectable in plasma in 6 animals at a mean concentration of 0.32 ± 0.10 μg/ml. Based on the MAICE principle and using the AIC test, the concentration-time curves were best fitted to a three compartmental model in 5 animals and a two compartmental model in 3 animals.

The distribution of PBZ in plasma was rapid in the first phase ($t_{\frac{1}{2}\alpha}$ = 0.22 ± 0.05 h) and much slower in the second phase ($t_{\frac{1}{2}\pi}$ = 5.35 ± 1.27 h). The elimination was very slow, indicated by a

$t_{\frac{1}{2}\beta}$ of 17.92 ± 1.74 h, a MRT of 21.63 ± 1.94 h, a slow Cl_B (4.56 ± 0.53 ml/kg.h) and small V_{ss} (98.66 ± 4.67 ml/kg) (Table 3.2. and Fig. 3.2).

The results analysed by non-compartmental modelling showed that PBZ distributed slowly into the tissue-cages although it could be detected by 2 h following administration. Similar values for C_{max} were achieved in exudate (22.32 ± 1.29) and transudate (22.07 ± 1.57) at 9.50 ± 0.73 h and 11.50 ± 1.92 h, respectively (Table 3.4). The elimination of PBZ from both tissue-cage fluids was slower than from plasma, indicated by the MRT values of 31.60 ± 1.84 h and 31.11 ± 2.41 h from exudate and transudate, respectively. This also indicated that PBZ had similar rates of penetration and elimination in both exudate and transudate (Table 3.4.). Before about 20 h PBZ concentrations in exudate and transudate were lower than in plasma and thereafter, the relative concentrations were reversed due to slow elimination from the cages (Fig. 3.2.).

The data for PBZ in tissue-cage fluids could be best fitted to a one compartment model with first order input and output and a lag time (R^2 > 0.95 for all data fitted). This illustrated that the elimination half lives ($t_{\frac{1}{2}k10}$) of PBZ were similar in plasma (17.92 ± 1.74 h), exudate (17.82 ± 1.27 h) and transudate (16.24 ± 1.60 h) (Tables 3.2, 3.3.). A lag time for PBZ of 1.66 ± 0.09 h in exudate and 1.89 ± 0.22 h in transudate indicated the slow penetration of PBZ from plasma into the tissue-cage fluids. The curves in Fig. 3.2. also showed that 20 h after drug administration, PBZ concentration declined at the similar rates in plasma, exudate and transudate although the concentrations of PBZ in exudate and transudate were slightly higher than in plasma at most of the time points.

The AUC ratios of exudate and transudate to plasma were high, 84.74 ± 5.91 % and 79.98 ± 4.53 %, respectively. After correction for MRT, both ratios became smaller: 56.76 ± 3.20 % for exudate to plasma and 54.55 ± 3.50 % for transudate to plasma. Pre- or post-correction by MRT, the AUC ratios of exudate to plasma were larger than transudate to plasma (Table 3.6.).

As described in Table 3.5. and Fig. 3.3., the metabolism of PBZ to OPBZ was very slow and weak, indicated by the low C_{max} values for OPBZ of 1.35 ± 0.16, 0.88 ± 0.14 and 0.82 ± 0.11 μg/ml in plasma, exudate and transudate, respectively. The AUC ratios of OPBZ to PBZ were also very small, about 4.5 % in plasma, exudate and transudate.

Table 3.2. Pharmacokinetic parameters of PBZ and FM in plasma following i.v. administration of PBZ (4.4 mg/kg) and FM (1.1 mg/kg) in sheep using compartmental modelling (n=8, mean ± SE)

Parameters	PBZ	FM
A (μg/ml)	45.95 ± 2.88	16.71 ± 1.49
P (μg/ml)	20.40 ± 4.01	NA
B (μg/ml)	31.62 ± 4.24	7.80 ± 1.16
α (h^{-1})	4.14 ± 0.97	3.38 ± 0.67
π (h^{-1})	0.1674 ± 0.0415	NA
β (h^{-1})	0.0405 ± 0.0045	0.31 ± 0.03
C_p^0 (μg/ml)	97.96 ± 3.63	24.51 ± 2.60
V_c (ml/kg)	45.15 ± 1.59	49.47 ± 6.40
K_{21} (h^{-1})	2.19 ± 0.47	1.29 ± 0.27
K_{31}(h^{-1})	0.13 ± 0.03	NA
K_{10} (h^{-1})	0.10 ± 0.01	0.83 ± 0.08
K_{12} (h^{-1})	1.87 ± 0.50	1.58 ± 0.36
K_{13} (h^{-1})	0.06 ± 0.02	NA
$k_{10\frac{1}{2}}$	7.27 ± 0.91	0.87 ± 0.06
$t_{\frac{1}{2}}\alpha$	0.22 ± 0.05	0.24 ± 0.03
$t_{\frac{1}{2}}\pi$	5.35 ± 1.27	NA
$t_{\frac{1}{2}}\beta$	17.92 ± 1.74	2.33 ± 0.18
$AUC_{0-\infty}$ (μg.h/ml)	946.23 ± 96.82	30.16 ± 3.25
$AUMC_{0-\infty}$ (μg.h^2/ml)	23517.5 ± 3996.4	83.12 ± 9.80
$MRT_{0-\infty}$ (h)	21.63 ± 1.94	2.78 ± 0.20
Cl_B (ml/kg/h)	4.56 ± 0.53	39.67 ± 4.27
V_{ss} (ml/kg)	98.67 ± 4.67	111.50 ± 17.08

Parameters for PBZ were derived from a three compartmental model in 5 sheep and data of FM were fitted to a two compartmental model.

Table 3.3. Pharmacokinetic parameters of PBZ in exudate and transudate following i.v. administration of PBZ at 4.4 mg/kg in sheep using a one compartment model with first order input and output and a lag time (n=8, mean ± SE)

Parameters	Exudate	Transudate
K_{01} (h^{-1})	0.36 ± 0.08	0.28 ± 0.07
K_{10} (h^{-1})	0.0402 ± 0.0027	0.0459 ± 0.0048
Lag time (h)	1.66 ± 0.09	1.89 ± 0.22
$AUC_{0-\infty}$ (μg.h/ml)	764.8 ± 76.2	718.0 ± 77.6
$t_{\frac{1}{2}k01}$ (h)	2.71 ± 0.69	3.44 ± 0.62
$t_{\frac{1}{2}k10}$ (h)	17.82 ± 1.27	16.24 ±1.60
t_{max} (h)	9.05 ± 0.80	11.26 ± 1.46
C_{max} (μg/ml)	21.28 ± 1.25	20.72 ± 1.55

Table 3.4. Pharmacokinetic parameters of PBZ in plasma, exudate and transudate following i.v. administration of PBZ at 4.4 mg/kg in sheep using non-compartmental modelling (n = 8, mean ± SE)

Parameters	Plasma	Exudate	Transudate
$C_{max\,(obs)}$ (μg/ml)	77.38 ± 5.45 ($C_p^{5\,min}$)	22.32 ± 1.29	22.07 ± 1.57
t_{max} (h)	NA	9.50 ± 0.73	11.50 ± 1.92
β (h^{-1})	0.0422 ± 0.0036	0.0254 ± 0.0013 $^{(n=7)}$	0.0222 ± 0.0059
$t_{\frac{1}{2}\beta}$	17.12 ± 1.20	27.72 ± 1.33 $^{(n=7)}$	31.22 ± 8.30
AUC_{0-last} (μg.h/ml)	963.54 ± 95.35	804.01±77.64	761.21 ± 77.53
$AUC_{0-\infty}$ (μg.h/ml)	970.32 ± 97.61	829.44 ± 89.12 $^{(n=7)}$	791.09 ± 91.83
$AUMC_{0-last}$ (μg.h^2/ml)	21499.39 ± 3623.75	26063.60 ± 3435.99	24695.25 ± 4265.92
$AUMC_{0-\infty}$ (μg.h^2/ml)	22668.53 ± 4037.94	28745.3 ± 4312.2 $^{(n=7)}$	34067.17 ± 9522.92
MRT_{0-last} (h)	21.22 ± 1.73	31.60 ± 1.84	31.11 ± 2.41
$MRT_{0-\infty}$ (h)	22.07 ± 2.01	33.68 ± 2.54 $^{(n=7)}$	39.19 ± 5.60
MAT_{0-last} (h)	NA	10.38 ± 0.99	9.90 ± 1.39
$MAT_{0-\infty}$ (h)	NA	11.81 ± 1.73$^{(n=7)}$	17.24 ± 4.58

β in exudate and transudate was calculated for the estimation of $AUC_{last-\infty}$ and $AUMC_{last-\infty}$ using the curve stripping method.

Table 3.5. Pharmacokinetic parameters of OPBZ in plasma, exudate and transudate following i.v. administration of PBZ at 4.4 mg/kg in sheep using non-compartmental modelling (n=8, mean ± SE)

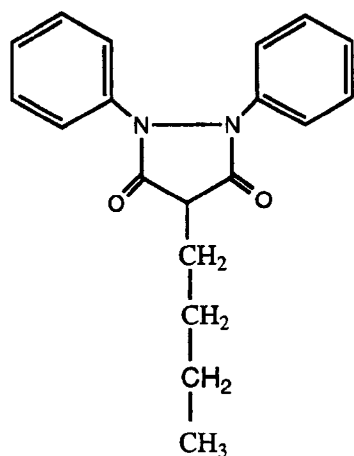
Parameters	Plasma	Exudate	Transudate
$C_{max\ (obs)}$ (μg/ml)		0.88 ± 0.14	0.82 ± 0.11
t_{max} (h)	9.88 ± 5.49	18.00 ± 4.90	14.50 ± 2.13
β	0.0265 ± 0.0014	0.0652 ± 0.00427 $^{(n=5)}$	0.0164 ± 0.0019
$t_{\frac{1}{2}\beta}$	26.22 ± 1.63	38.34 ± 10.58 $^{(n=5)}$	46.79 ± 7.45
AUC_{0-last} (μg.h/ml)	40.75 ± 4.76	32.43 ± 4.42	34.13 ± 2.71
$AUC_{0-\infty}$ (μg.h/ml)	41.58 ± 4.71	33.25 ± 4.24 $^{(n=5)}$	35.79 ± 2.90
$AUMC_{0-last}$ (μg.h^2/ml)	1238.4 ± 206.9	1199.0 ± 277.7	1406.4 ± 75.4
$AUMC_{0-\infty}$ (μg.h^2/ml)	1373.9 ± 215.1	1444.6 ± 542.1 $^{(n=5)}$	1791.9 ± 203.7
MRT_{0-last} (h)	29.63 ± 2.07	35.23 ± 2.94	43.04 ± 4.12
$MRT_{0-\infty}$ (h)	33.10 ± 2.74	39.16 ± 8.68 $^{(n=5)}$	53.00 ± 9.32
MAT_{0-last} (h)	NA	5.60 ± 2.96	13.40 ± 2.84

The parameters were derived from non-compartmental modelling, where β was calculated for estimation of $AUC_{last-\infty}$ and $AUMC_{last-\infty}$ using curve the stripping method.

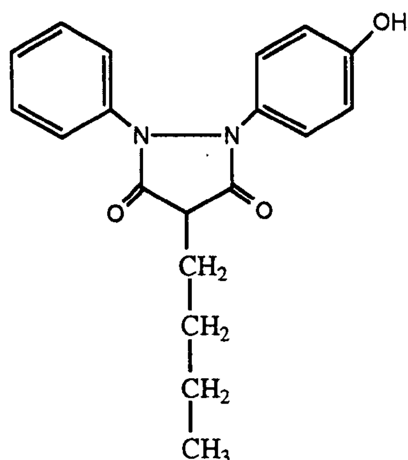
Table 3.6. Mean ± SE AUC ratios of PBZ, OPBZ and FM, in plasma, exudate and transudate following FM (1.1 mg/kg) or PBZ (4.4 mg/kg) given i.v. in 8 sheep.

Parameters	PBZ	OPBZ	FM
AUC ratio (%)			
Exudate:plasma	84.74 ± 5.91	81.95 ± 9.62	92.10 ± 8.00
Transudate:plasma	79.98 ± 4.53	87.86 ± 7.23	97.93 ± 10.00
AUC ratio (%, OPBZ:PBZ)			
Plasma	4.53 ± 0.67	N/A	N/A
Exudate	4.22 ± 0.54	N/A	N/A
Transudate	4.72 ± 0.53	N/A	N/A
AUC ratio (%, Corrected by MRT)			
Exudate:Plasma	56.76 ± 3.20	66.65 ± 3.13	23.09 ± 2.03
Transudate:Plasma	54.55 ± 3.50	61.09 ± 5.62	20.59 ± 2.71

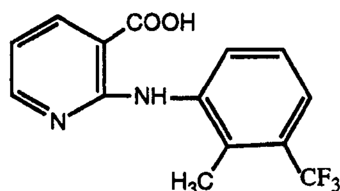
The ratios were calculated using AUC_{0-last}.



Phenylbutazone



Oxyphenbutazone



Flunixin

Fig 3.1. Chemical structure of phenylbutazone and flunixin

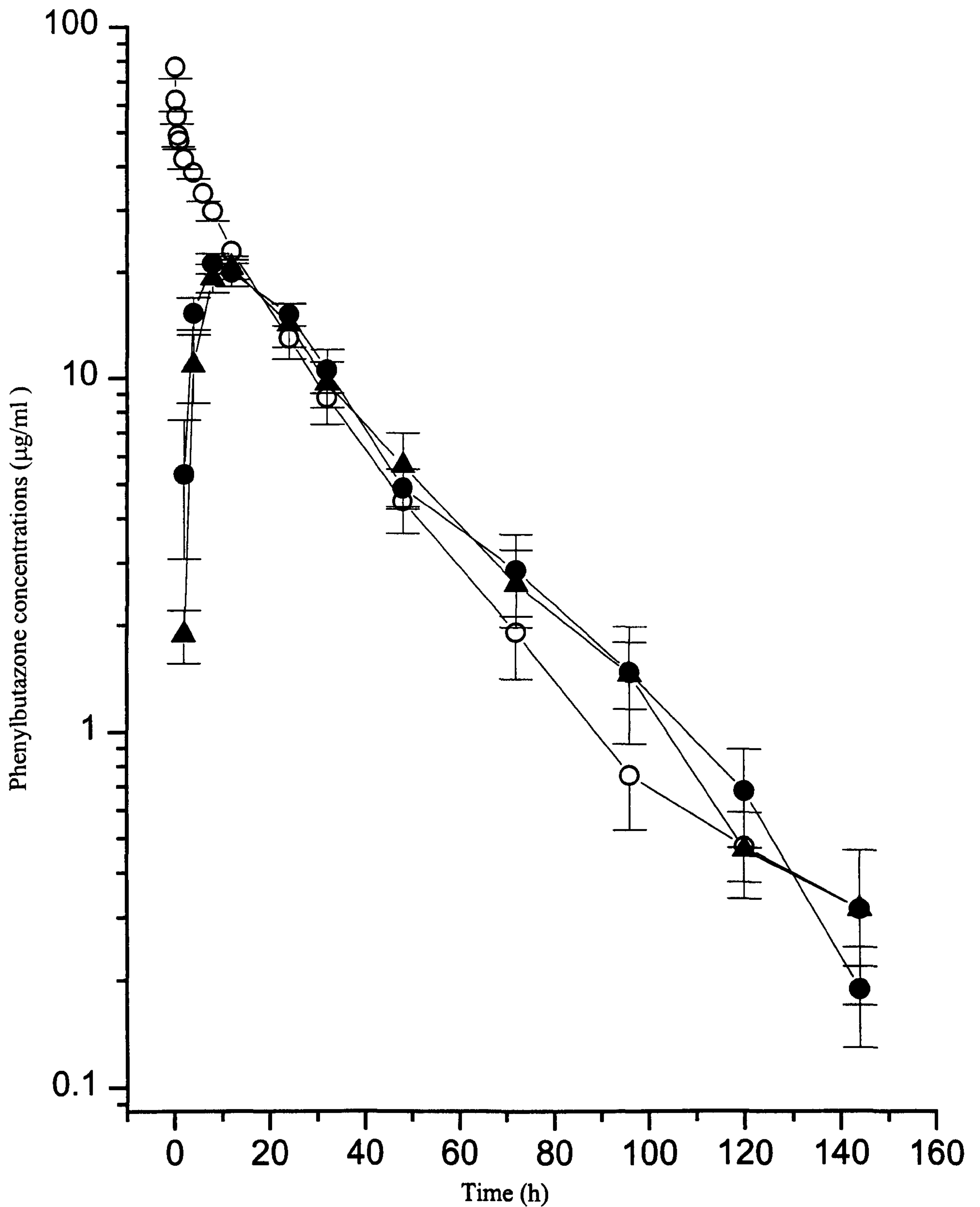


Fig.3.2. Concentration versus time semilogarithmic plot of PBZ in plasma (–○–), exudate (–●–) and transudate (–▲–) in sheep (n=8, mean ± SE) following PBZ administration i.v. at 4.4 mg/kg.

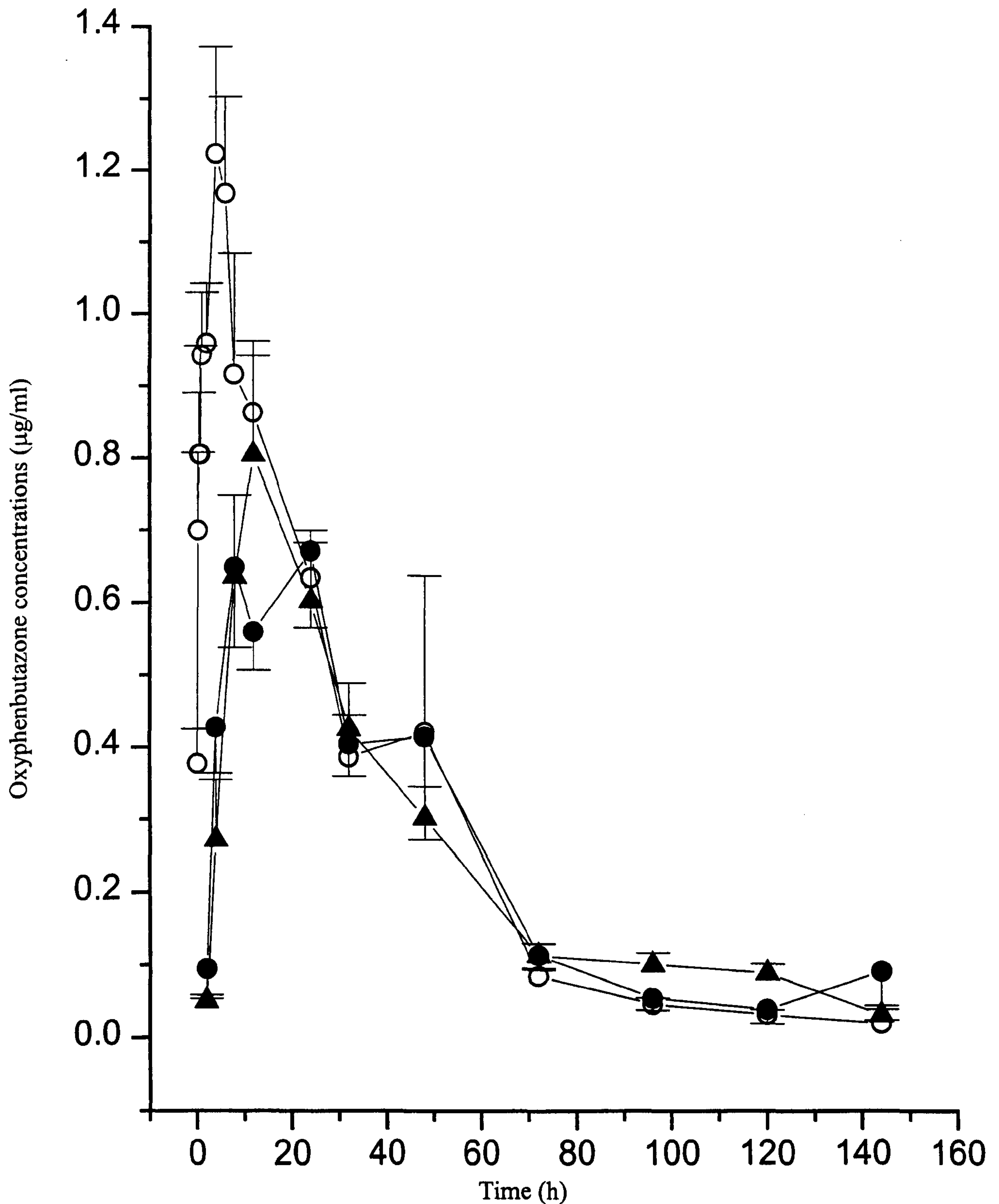


Fig 3.3. Concentration versus time plot of OPBZ in plasma (–○–), exudate (–●–) and transudate (–▲–) following administration of PBZ i.v. at 4.4 mg/kg in sheep (n=8, mean ± SE).

3.4.1.2. Flunixin meglumine

Pharmacokinetic data for FM in plasma, exudate and transudate following FM administered i.v. at 1.1 mg/kg in sheep are presented in Tables 3.2., 3.6., 3.7. and 3.8., and Fig. 3.4.

Following i.v. administration of FM at a single dose rate of 1.1 mg/kg in the sheep, plasma concentration declined much more rapidly than PBZ (Fig. 3.4.). Concentrations were detectable only for up to 12 h in 5 animals and up to 24 h in 3 animals. Based on the MAICE principle using the AIC test, the concentration-time data were best fitted to a two compartmental model in 7 sheep and to a three compartmental model in 1 sheep.

Distribution and elimination of FM in plasma were fast, indicated by a $t_{\frac{1}{2}\alpha}$ of 0.24 ± 0.03 h, an $t_{\frac{1}{2}\beta}$ of 2.33 ± 0.18 h and a MRT of 2.78 ± 0.20 h. Volume of distribution at steady state was small (111.50 ± 17.08 ml/kg). This led to a slow Cl_B, 39.67 ± 4.27 ml/kg/h in spite of the elimination half life (Table 3.2.).

Flunixin meglumine was detectable in exudate and transudate 2 h after administration. During the first 8 h the concentrations in exudate and transudate were lower than in plasma. After this the concentrations in exudate and transudate were higher than in plasma. Drug concentrations were measurable up to 32 h in exudate and 48 h in transudate in most of the animals. The PK analyses showed that the elimination of FM from tissue-cages was much slower than from plasma, indicated by a MRT of 12.98 ± 1.02 h for exudate and 15.35 ± 0.65 h for transudate whereas MRT in plasma was only 3.20 ± 0.19 h (Table 3.7. and Fig. 3.4.).

The penetration of FM into the tissue cages was very slow indicated by a MAT of 9.79 ± 0.93 h for exudate and 12.15 ± 0.70 h for transudate. A C_{max} of 1.82 ± 0.22 μg/ml in exudate occurred at 5.50 ± 0.73 h and a C_{max} of 1.58 ± 0.30 μg/ml in transudate at 8.00 ± 0.76 h. During the period while FM was distributing into tissue-cages, the concentrations in exudate were higher than in transudate, however after C_{max} had been achieved these observations were reversed. The data in individual animals indicated that in cages with a faster drug penetration rate and a higher C_{max} the drug was also more quickly eliminated (Table 3.7. and Fig. 3.4.).

The concentration-time data of FM in tissue-cage fluids could be best fitted to a one compartment model with first order input and output, and a lag time and the parameters are given in Table 3.8. A lag time for FM of 1.60 ± 0.06 h in exudate and 1.67 ± 0.12 h in transudate illustrated a slow extravascular penetration. The values of $t_{\frac{1}{2}k10}$ in exudate (8.53 ± 1.04 h) and transudate (11.96 ± 1.07 h) were longer than the $t_{\frac{1}{2}\beta}$ in plasma (2.33 ± 0.18 h). These results (Table 3.8.) were consistent with the results analysed by non-compartment modelling (Table 3.7.).

The AUC ratios of exudate AUC and transudate AUC to plasma AUC were higher than those obtained for PBZ, 92.0 ± 8.0 and 97.1 ± 10 %, respectively. Following correction for MRT, the AUC ratios were smaller (half those of PBZ) and the ratio of exudate to plasma was larger than transudate to plasma (Table 3.6.).

Table 3.7. Pharmacokinetic parameters of FM in plasma, exudate and transudate following i.v. administration of FM at 1.1 mg/kg in sheep using non-compartmental modelling (n=8, mean±SE)

Parameters	Plasma	Exudate	Transudate
$C_{max\,(obs)}$ (μg/ml)	20.48 ± 1.96 (C_p^{5min})	1.82 ± 0.22	1.58 ± 0.30
t_{max} (h)	NA	5.50 ± 0.73	8.00 ± 0.76
β (h^{-1})	0.2548 ± 0.0290	0.0577 ± 0.0244 $^{(n=3)}$	0.0885 ± 0.0545 $^{(n=5)}$
$t_{\frac{1}{2}\beta}$	2.93 ± 0.27	16.4543 ± 5.4666 $^{(n=3)}$	21.85 ± 2.59 $^{(n=5)}$
$AUC_{0\text{-}last}$ (μg.h/ml)	30.56 ± 3.41	27.38 ± 2.88	29.47 ± 4.72
$AUC_{0\text{-}\infty}$ (μg.h/ml)	30.60 ± 3.42	26.71 ± 1.59 $^{(n=3)}$	39.53 ± 8.54 $^{(n=5)}$
$AUMC_{0\text{-}last}$ (μg.h^2/ml)	99.00 ± 13.60	356.51 ± 47.03	457.73 ± 78.54
$AUMC_{0\text{-}\infty}$ (μg.h^2/ml)	100.86 ± 14.47	347.29 ± 59.86 $^{(n=3)}$	1054.6 ± 245.1 $^{(n=5)}$
$MRT_{0\text{-}last}$ (h)	3.20 ± 0.19	12.98 ± 1.02	15.35 ± 0.65
$MRT_{0\text{-}\infty}$ (h)	3.24 ± 0.20	13.15 ± 2.43 $^{(n=3)}$	26.12 ± 1.40 $^{(n=5)}$
$MAT_{0\text{-}last}$ (h)	NA	9.79 ± 0.93	12.15 ± 0.70
$MAT_{0\text{-}\infty}$ (h)	NA	9.59 ± 2.58$^{(n=3)}$	22.99 ± 1.18 $^{(n=5)}$

β in exudate and transudate was calculated for the estimation of $AUC_{last\text{-}\infty}$ and $AUMC_{last\text{-}\infty}$ using the curve stripping method.

Table 3.8. Pharmacokinetic parameters of FM in exudate and transudate following i.v. administration of FM at 1.1 mg/kg in sheep using a one compartment model with first order input and output and a lag time (n=8, mean ± SE).

Parameters	Exudate	Transudate
K_{01} (h^{-1})	0.91 ± 0.18	0.99 ± 0.23
K_{10} (h^{-1})	0.0925 ± 0.0141	0.0613 ± 0.0054
Lag time (h)	1.60 ± 0.06	1.67 ± 0.12
$AUC_{0\text{-}\infty}$ (μg.h/ml)	27.29 ± 3.19	28.92 ± 4.62
$t_{\frac{1}{2}k01}$ (h)	0.94 ± 0.14	0.93 ± 0.15
$t_{\frac{1}{2}k10}$ (h)	8.53 ± 1.04	11.96 ± 1.07
t_{max} (h)	4.50 ± 0.45	5.23 ± 0.45
C_{max} (μg/ml)	1.80 ± 0.24	1.40 ± 0.24

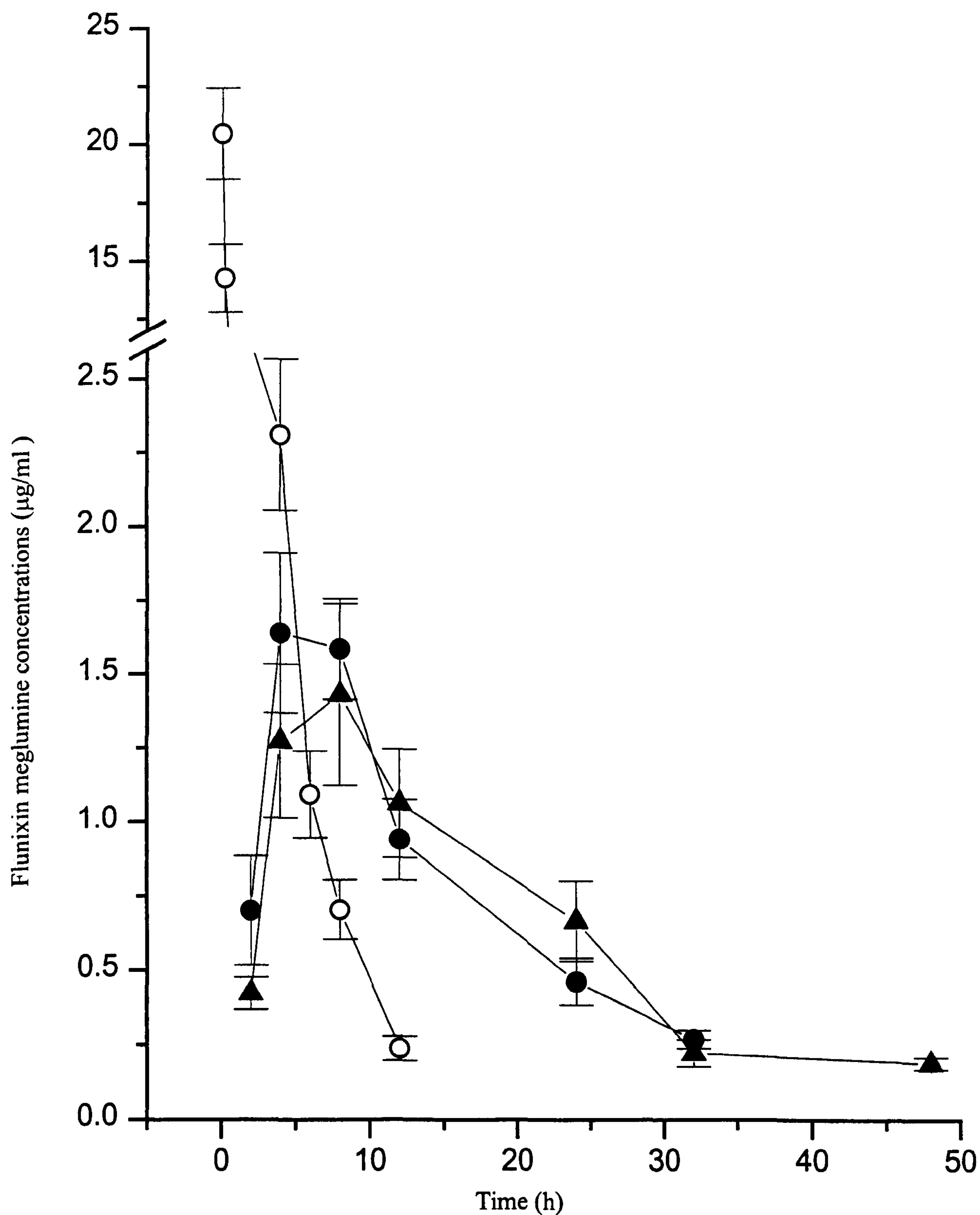


Fig. 3.4. Concentration versus time plot of FM in plasma (–○–), exudate (–●–) and transudate (–▲–) following i.v. administration of FM at 1.1 mg/kg in sheep (n=8, mean ± SE).

3.4.2. Pharmacodynamics

3.4.2.1. The effects of PBZ and FM on inflammatory skin temperature

The changes in skin temperature (°C) over the cages (expressed by the differences between inflamed and non-inflamed tissue-cages) are illustrated in Table 3.9.

Intracaveal injection of carrageenan led to an increase in skin temperature over the treated cages. In the PLB-treated group, the overall mean temperature increased by 0.58 ± 0.12 °C over a period of 144 h, with a maximum increase of 1.52 ± 0.33 °C recorded at 4 h. Most of the values recorded at each time point were higher than the pre-values and compared with pre-values, a significant increase was achieved at 2, 4 and 12 h ($P<0.05$).

In the PBZ-treated group, the temperature was higher than the pre-values and PLB-treated group at 1 h ($P<0.05$) and then returned to the pre-values. The temperatures were slightly lower than the PLB-treated group in some time points, but no significant differences were achieved ($P>0.05$). In the FM-treated group, there was no significant change in the temperature at any time compared to pre-induction values. The temperature was significantly lower than that in the PLB-treated group at 2, 4 and 6 h ($P<0.05$) and it was also lower than in the PBZ-treated group at 1 and 4 h ($P<0.05$).

The analysis also illustrated that the pre-values and ambient temperature did not exert significant influence on the changes caused by carrageenan stimulation and drug administration ($P>0.05$) and that the difference between cross-over periods was not significant ($P>0.05$). However the differences of individual sheep and treatment × sheep interaction (the response of the sheep to treatments) were significant ($P<0.05$).

3.4.2.2. The effects of PBZ and FM on serum TXB_2 generation

Mean ± SE serum TXB_2 concentrations following i.v. administration of PLB, PBZ and FM in the sheep are presented in Fig. 3.5. The percentage inhibition of serum TXB_2 against plasma drug concentrations is illustrated in Table 3.10. and the PD modelling results are given in Table 3.11.

There were significant differences in serum TXB_2 concentration between the overall means of the three treatment groups ($P<0.01$). The interaction of treatment × time was significantly different ($P<0.01$), indicating that the measurements at each time point were significantly different between treatment groups.

Fisher's multiple comparisons were carried out to confirm the source of differences. Compared with the pre-values, serum TXB_2 generation was attenuated in the PLB-treated group for 12 h ($P<0.05$). The concentrations were higher than the pre-values after 72 h but they were not significantly different ($P>0.05$). Compared with the pre-values and the PLB-treated group, PBZ inhibited TXB_2 generation for up to 32 h ($P<0.05$) although the inhibition was partial. From 48 h post PBZ administration, the generation of TXB_2 was completely restored and on occasion, TXB_2 concentrations were higher than pre-treatment ($P<0.05$). By applying a Sigmoid inhibitory effect model to the PBZ treated group, E_{max} was estimated to be 79.94 ± 6.20 % despite high plasma concentrations of PBZ (C_p^0 of PBZ was 97.96 ± 3.63 μg/ml). An IC_{50} of 10.86 ± 1.81 μg/ml occurred 29.96 ± 2.62 h after PBZ treatment. In the FM-treated group, serum TXB_2 generation was abolished for up to 8 h although FM concentration at 8 h was 0.70 ± 0.28 μg/ml. Compared with the pre-values and the PLB-treated group, a significant inhibition was observed for up to 32 h ($P<0.05$) after FM administration. After this TXB_2 generation was restored and did not differ significantly from the pre-values or PLB-treated group ($p>0.05$). The E_{max} was 99.96 ± 4.0 % and IC_{50} was 0.0053 ± 0.0032 μg/ml occurring at 28.96 ± 1.86 h. The percentage inhibition of TXB_2 was greater than that in the PBZ-treated group for up to 24 h after drug administration ($p<0.05$).

The pre-values had significant effects on the inhibitory effects of the drugs ($r = -0.34 \pm 0.10$, $p = 0.001$). This suggests a competitive inhibitory nature of the drugs for COX in PLT. There were

significant differences of TXB_2 inhibition with time, sheep, periods and their two way interactions ($P<0.05$). This may reflect that a number of factors affected the experiments.

3.4.2.3. The effects of PBZ and FM on carrageenan-induced PGE_2 generation

The effects of PLB, PBZ and FM on PGE_2 generation in carrageenan-induced exudate are demonstrated in Tables 3.11. and 3.12. and Fig. 3.6.

Prostaglandin E_2 was not detectable in tissue-cage fluid (below the limit of quantification of 0.8 ng/ml) before injection of carrageenan. In the PLB-administered group, PGE_2 concentration rose steadily to a C_{max} of 35.65 ± 4.92 ng/ml achieved at 12 h which was 4 h after the second injection of carrageenan. The changes in exudate PGE_2 were time-related ($P<0.001$) and this indicated a reversible acute inflammatory response. Prostaglandin E_2 in the PLB-treated group was detectable over a period of 2-144 h and was significantly higher than the pre-values in the exudate from 4 to 120 h following the injection of carrageenan ($P<0.05$). Exudate PGE_2 concentrations in PBZ-treated group were significantly higher than pre-values from 8 to 48 h but no significant generation of exudate PGE_2 were observed in FM-treated group at any time point compared with the pre-values ($P>0.05$).

There were significant differences of overall mean exudate PGE_2 concentrations between the PLB (16.32 ± 1.70 ng/ml) and treatment groups of PBZ (10.41 ± 1.55 ng/ml) and FM (1.72 ± 0.43 ng/ml) during a period of 144 h ($P<0.01$). The interactions of treatment × time and treatment × sheep were significantly different ($P<0.001$) and the concentrations of exudate PGE_2 were different between individual animals ($P<0.001$).

Phenylbutazone attenuated PGE_2 generation over a 144 h period, however the inhibition was weak and statistical significance was achieved only at 4 and 12 h ($P<0.05$) when compared with the PLB-treated group. In exudate, the C_{max} for PBZ of 22.32 ± 1.29 μg/ml produced only 10 % inhibition of PGE_2 generation. Extrapolation suggested an exudate PBZ concentration > 100 μg/ml would be required to produce an IC_{50} for exudate PGE_2 generation.

Flunixin meglumine abolished exudate PGE_2 synthesis for up to 4 h and inhibited the PGE_2 generation significantly from 8 h to 32 h compared with PLB-treated group ($p<0.05$). The inhibitory effects of FM were considerably greater than those of PBZ, and PGE_2 concentrations in tissue cages from the FM-treated group were significantly lower than in PBZ-treated group up to 32 h after drug administration ($P<0.05$). After 32 h the inhibition was not statistically significant ($p>0.05$) compared with the PLB-treated group although the concentrations of PGE_2 in the FM-treated group were much lower than in the PLB-treated group. Flunixin meglumine produced greater than 50 % of the PGE_2 inhibitory effect in all animals up to 144 h at which time the last samples were taken. Simulation using the exudate PK equation for FM suggested that the drug concentration in exudate at 144 h would have been about 0.00019 ± 0.00012 µg/ml. Pharmacodynamic modelling was considered inapplicable to the PBZ-treated group due to the weak effect of PBZ on PGE_2 generation.

Significant differences in exudate PGE_2 concentrations were also observed between experimental sequences and cross-over periods ($P<0.001$). The capacity of carrageenan-induced exudate PGE_2 generation in the cages was significantly correlated with the treatment results ($r = -0.33 \pm 0.01$, $P<0.01$) and this may indicate that high drug concentrations were required to inhibit the high concentration of the PGE_2 and also indicates the competitive inhibitory nature of the NSAIDs.

3.4.2.4. The effects of PBZ and FM on exudate LTB_4 generation

Concentrations of LTB_4 (mean ± SE) in exudate following i.v. administration of PLB, PBZ and FM are given in Table 3.13.

Following the intracaveal injection of 1 % of the mild irritant carrageenan, exudate LTB_4 generation in all treatment groups increased significantly over a 120 h period ($P<0.05$). The maximal concentration was 1.63 ± 0.31 ng/ml recorded at 12 h in the PLB-treated group, 1.71 ± 0.52 ng/ml at 72 h in the PBZ-treated group and 1.60 ± 0.28 ng/ml at 8 h in FM-treated group. The overall means of exudate LTB_4 concentration in PBZ-treated (1.37 ± 0.10 ng/ml) and FM-treated (1.40 ± 0.08 ng/ml) groups were slightly higher than in the PLB-treated (1.26 ± 0.10 ng/ml) group but the differences were not significant ($P>0.05$).

The overall treatment × time interaction was not significantly different (P>0.05). However significant difference was found at some time points when using Fisher's comparisons. Compared with the PLB-administered group a significant increase in exudate LTB_4 generation was obtained at 48 h in the PBZ-treated group and at 24 and 48 h in the FM-treated group (P<0.05).

There were significant differences in exudate LTB_4 generation in experimental sequences, cross-over periods, time, sheep, and treatment × sheep interaction (P<0.001).

3.4.2.5. The effects of PBZ and FM on exudate WBC accumulation

Following the intracaveal administration of carrageenan, the number of WBC in exudate increased significantly at 4, 8, 12, 24 and 48 h in the PLB-administered group, and at 8, 12 and 24 h in the PBZ and FM treated groups (P<0.05). The maximal numbers of WBC (10^9 cell/L) were 41.96 ± 8.59 in the PLB treated group, 26.60 ± 5.42 in the PBZ-treated group and 37.49 ± 7.77 in the FM-treated group, and all occurred at 24 h. The overall mean cell numbers in the PBZ-treated group (14.79 ± 79 × 10^9 cell/L) and FM-treated group (16.10 ± 2.45 × 10^9 cells/L) were lower than in the PLB-treated group (22.29 ± 3.00 × 10^9 cells/L) but they were not significantly different (P>0.05) due to a large SE. For measurements at each time point, the numbers of exudate WBC in the FM-treated group was significantly lower than in the PLB-treated group at 12 h. The ANOVA confirmed that there were significant differences in WBC numbers in individual animals (P<0.05) and the response to the treatments among the individual animals was also different (P<0.01). (Fig 3.7.).

3.4.2.6. The effects of PBZ and FM on WBC and PLT in blood

The numbers of WBC and PLT in blood were not significantly modified by PBZ or FM at any sampling time (p>0.05, Fig. 3.8.).

Table 3.9. The effects of PBZ and FM on inflammatory skin temperature

Time (h)	Placebo	Phenylbutazone	Flunixin
Pre-values	-0.31 ± 0.46	-0.21 ± 0.84	-0.04 ± 0.44
1	-0.55 ± 0.62	1.28 ± 0.40[ac]	-0.03 ± 0.52[b]
2	1.20 ± 0.44[c]	-0.31 ± 0.40	-0.20 ± 0.67[a]
4	1.52 ± 0.33[c]	0.76 ± 0.47	-0.96 ± 0.43[ab]
6	0.91 ± 0.37	0.71 ± 0.52	-0.57 ± 0.44[a]
8	0.35 ± 0.54	0.18 ± 0.31	-0.01 ± 0.34
12	1.06 ± 0.45[c]	-0.01 ± 0.35	0.54 ± 0.40
24	0.40 ± 0.39	0.65 ± 0.59	0.97 ± 0.32
32	0.30 ± 0.45	0.32 ± 0.72	0.36 ± 0.43
48	0.87 ± 0.41	0.51 ± 0.51	0.78 ± 0.46
72	0.26 ± 0.38	-0.30 ± 0.49	0.80 ± 0.48
96	0.65 ± 0.51	0.45 ± 0.49	0.36 ± 0.44
120	0.60 ± 0.31	0.40 ± 0.42	0.37 ± 0.30
144	0.50 ± 0.38	0.71 ± 0.41	0.18 ± 0.72

All values (°C) are mean ± SE in 8 sheep. The values are the differences of the temperature between inflamed and non-inflamed tissue-cages (the temperature over inflamed tissue-cage minus the temperature over non-inflamed tissue-cages). (a) $P<0.05$ compared with the PLB-treated group, (b) $P<0.05$ compared with the PBZ-treated group. and (c) $P<0.05$ compared with the control values.

Table 3.10. Relationship between plasma drug concentrations and percentage TXB_2 inhibition in serum following i.v. administration of PBZ (4.4 mg/kg) and FM (1.1 mg/kg) in sheep (n=8, mean ± SE)

Time (h)	PBZ-treated group		Flunixin-treated group	
	Drug conc (μg/ml)	Inhibition (%)	Drug conc (μg/ml)	Inhibition (%)
1	47.53 ± 2.73	73.14 ± 11.78 [ac]	6.79 ± 2.31	100.00 ± 0.00 [abc]
2	42.10 ± 2.65	81.29 ± 6.24 [ac]	3.87 ± 1.47	100.00 ± 0.00 [abc]
4	38.55 ± 1.64	67.65 ± 6.69 [ac]	2.31 ± 0.73	100.00 ± 0.00 [abc]
6	33.60 ± 1.66	76.92 ± 6.37 [ac]	1.09 ± 0.42	100.00 ± 0.00 [abc]
8	29.92 ± 1.90	74.57 ± 4.72 [ac]	0.70 ± 0.28	100.00 ± 0.00 [abc]
12	23.00 ± 1.75	72.82 ± 5.42 [ac]	0.24 ± 0.12	99.76 ± 0.24 [abc]
24	13.04 ± 1.69	39.14 ± 8.42 [ac]	0.05 ± 0.01	65.76 ± 7.27 [abc]
32	8.84 ± 1.43	48.96 ± 5.75 [ac]	UD	37.33 ± 4.35 [ac]
48	4.52 ± 0.86	6.89 ± 7.96	UD	6.46 ± 5.10
72	1.93 ± 0.51	-8.75 ± 5.93	UD	1.44 ± 8.73
96	0.76 ± 0.23	-7.24 ± 17.56	UD	-5.50 ± 4.96
120	0.48 ± 0.10	-26.10 ± 9.91	UD	3.01 ± 4.56
144	0.32 ± 0.10	-15.97 ± 7.93	UD	-5.70 ± 9.05

UD: undetectable (below the limit of quantification of 0.05 μg/ml). (a) P< 0.05, compared with the PLB-treated group, (b) P<0.05, compared with the PBZ-treated group and (c) P<0.05, compared with the group control (pre-treatment values).

Table 3.11. The effects of PBZ and FM on serum TXB_2 and exudate PGE_2 generation

	Thromboxane B_2		Prostaglandin E_2	
Parameters	PBZ	FM	PBZ	FM
E_{max} (%)	79.94 ± 6.20	99.96 ± 0.04	< 40	100
IT_{50} (h)	29.96 ± 2.62	28.96 ± 1.86	NA	> 144
IC_{50} (μg/ml)	10.86 ± 1.81	0.0053 ± 0.0032	>200	0.00019 ± 0.00012
γ	3.91 ± 0.37	4.59 ± 0.59	NA	NA
$AUC_{0\text{-}72h}$	2302.96 ± 356.22	3000.14 ± 288.80	NA	NA

All values are mean ±SE in 8 sheep. N/A = not applicable. AUC = area under the curve using percentage TXB_2 inhibition against time plot. * P<0.01 compared with the PBZ-treated group using a paired student *t* test (two tails).

Table 3.12. The effect of PBZ and FM on PGE_2 generation in carrageenan-induced inflamed exudate in sheep

Time (h)	PLB-treated group	PBZ-treated group	FM-treated group
Pre	ND	ND	ND
2	4.12 ± 2.57	0.18 ± 0.14	0.00 ± 0.00
4	15.00 ± 5.33	7.17 ± 3.21*	0.00 ± 0.00*
8	31.21 ± 3.99	28.90 ± 5.31	0.18 ± 0.18*#
12	35.65 ± 4.92	22.05 ± 5.12*	1.69 ± 1.45*#
24	29.07 ± 3.07	24.05 ± 5.77	3.18 ± 2.58*#
32	19.94 ± 4.67	14.29 ± 5.26	4.71 ± 2.59*#
48	8.67 ± 2.86	7.43 ± 4.01	2.10 ± 1.34
72	7.61 ± 4.21	1.60 ± 1.15	1.17 ± 0.48
96	7.72 ± 4.34	1.63 ± 0.93	1.69 ± 0.85
120	8.93 ± 4.79	1.56 ± 0.91	2.61 ± 0.98

All values are mean ± SE expressed in ng/ml in 8 sheep. N/D means undetectable (below the limit of quantification of 0.8 ng/ml). Flunixin meglumine at 1.1 mg/kg , PBZ at 4.4 mg/kg or PLB (0.9% saline) at the same volume as the drugs were given i.v. to the sheep at a single dose rate. (*) $P<0.05$ compared with the PLB-treated group and (#) $P<0.05$ compared with the PBZ-treated group.

Table 3.13. The effect of PBZ and FM on LTB_4 generation in carrageenan-induced inflamed exudate in sheep.

Time (h)	PLB-treated group	PBZ-treated group	FM-treated group
Pre	NA	NA	NA
2	1.02 ± 0.30	1.10 ± 0.30	1.10 ± 0.22
4	1.32 ± 0.31	1.20 ± 0.29	1.39 ± 0.26
8	1.33 ± 0.36	1.22 ± 0.25	1.60 ± 0.28
12	1.63 ± 0.31	1.43 ± 0.28	1.55 ± 0.25
24	1.08 ± 0.25	1.21 ± 0.32	1.48 ± 0.32*
32	1.18 ± 0.26	1.40 ± 0.35	1.38 ± 0.26
48	1.13 ± 0.24	1.46 ± 0.37*	1.45 ± 0.38*
72	1.47 ± 0.41	1.71 ± 0.52	1.26 ± 0.26#
96	1.48 ± 0.40	1.61 ± 0.42	1.51 ± 0.30
120	1.24 ± 0.32	1.46 ± 0.37	1.55 ± 0.31
144	0.76 ± 0.19	1.23 ± 0.28	0.65 ± 0.09

All values are mean ± SE expressed in ng/ml in 8 sheep. Flunixin meglumine at 1.1 mg/kg , PBZ at 4.4 mg/kg or PLB (0.9% saline) at the same volume as the drugs were given i.v. to the sheep as a single dose rate.(*) $P<0.05$ compared with the PLB-treated group and (#) $P<0.05$ compared with the PBZ-treated group.

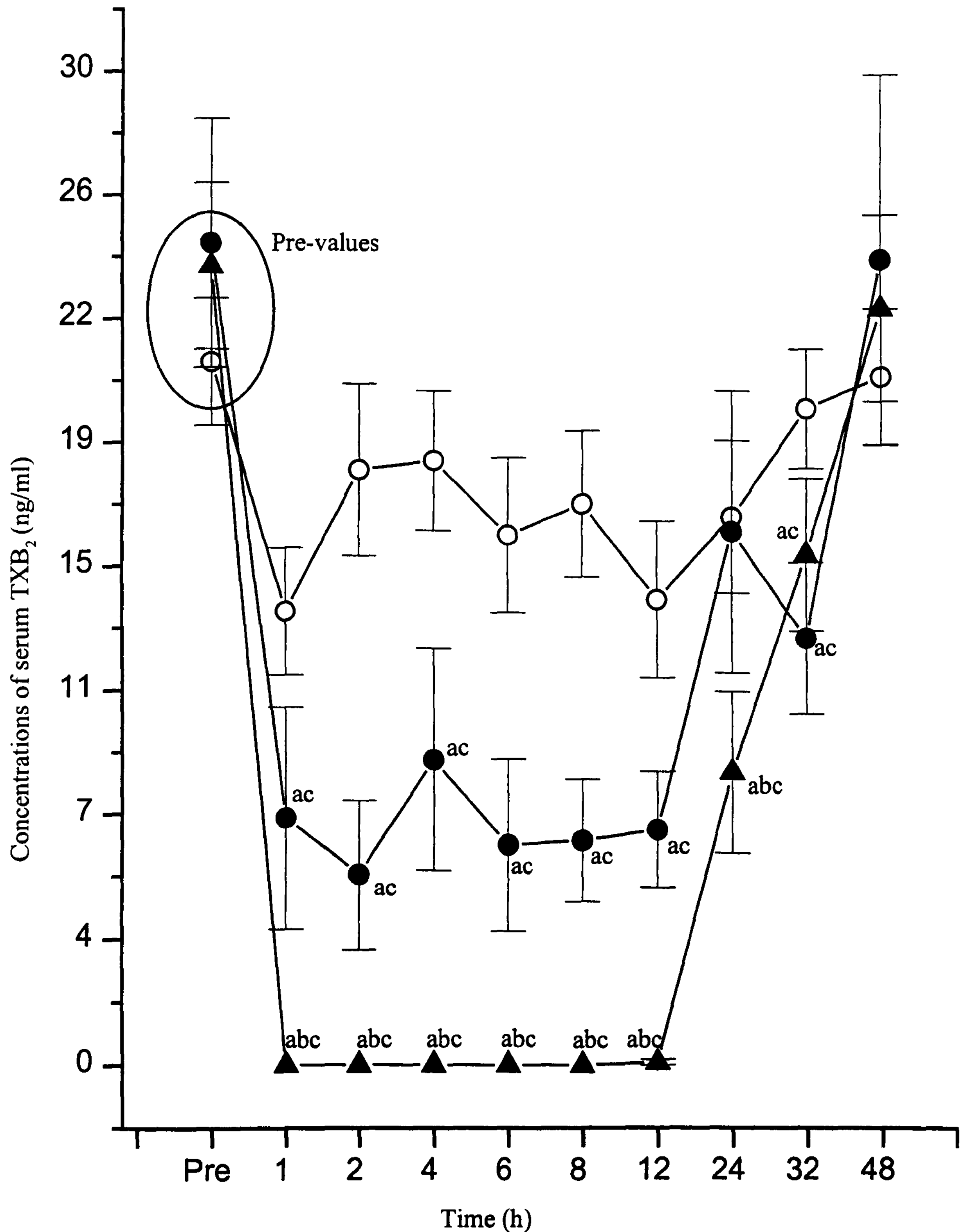


Fig 3.5. Effects of PBZ and FM on serum TXB_2. All values (ng/ml) are mean ± SE in 8 sheep. (–○–) PLB i.v. (–●–) PBZ i.v. at 4.4 mg/kg and (–▲–) FM i.v. at 1.1 mg/kg. (a) $P<0.05$, compared with placebo-treated group, (b) $P<0.05$, compared with PBZ-treated group and (c) $P<0.05$, compared with the group pre-treatment.

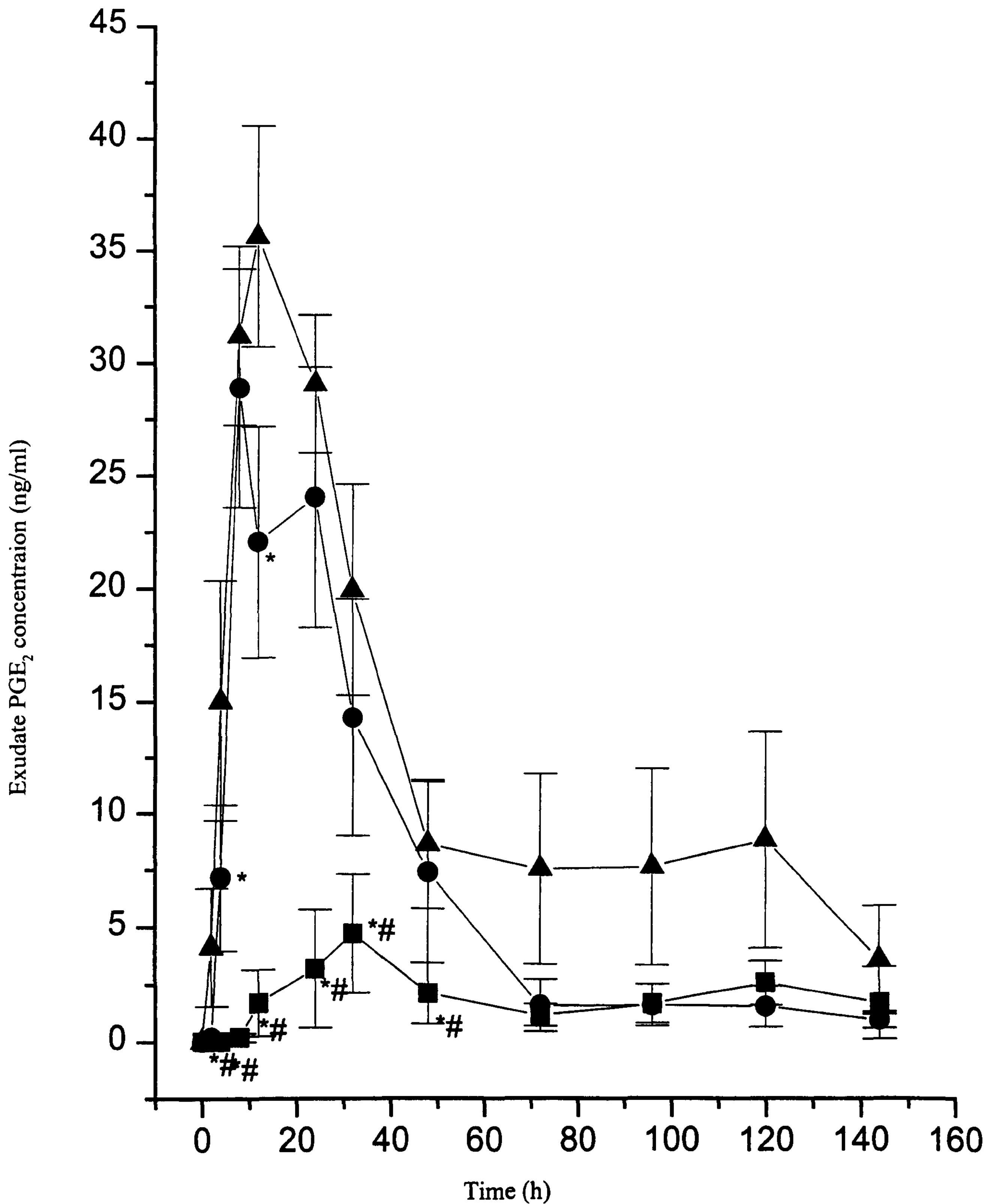


Fig 3.6. Effects of PBZ (–●–, 4.4 mg/kg, i.v.), FM (–■–, 1.1 mg/kg, i.v.) and PLB (–▲–, saline, i.v.) on carrageenan-induced PGE_2 generation in exudate in sheep (n=8, mean ± SE). (*) P<0.05, compared with the PLB-treated group and (#) P<0.05, compared with the PBZ-treated group.

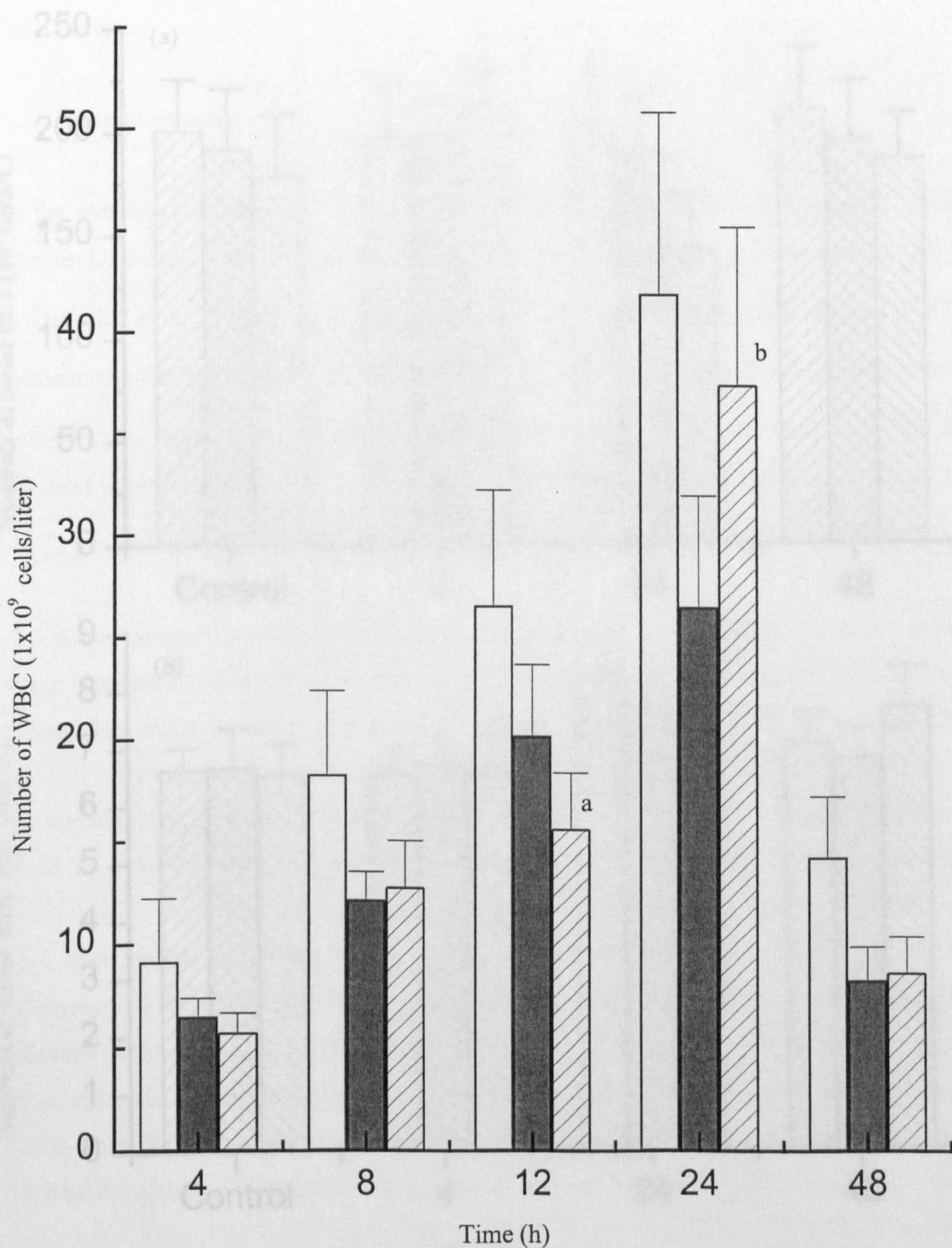


Fig 3.7. Effects of PBZ and FM on the recruitment of WBC in inflamed exudate. All values ($1x10^9$ cells/litre) are mean ± SE in 8 sheep. ( ) PLB i.v., ( ) PBZ i.v. at 4.4 mg/kg and ( ) FM i.v. at 1.1 mg/kg. (a) $P<0.05$, compared with PLB-treated group and (b) $P<0.05$, compared with PBZ-treated group.

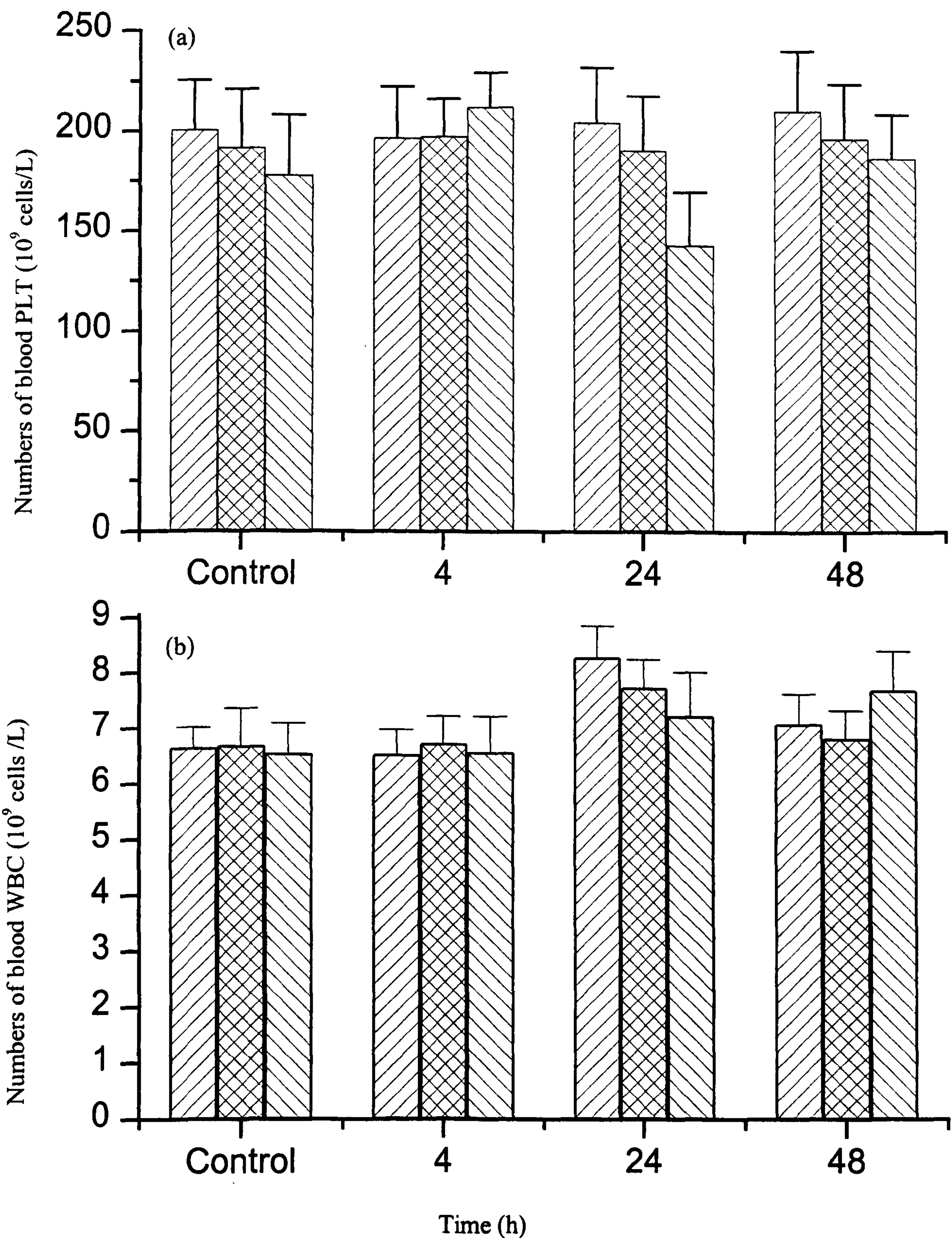


Fig 3.8. Effects of PLB i.v. ( ), PBZ i.v. at 4.4 mg/kg ( ) andFM i.v. at 1.1 mg/kg ( ) on venous blood PLT (a) and WBC (b) Values (10^9 cells/L) are expressed in mean ± SE in 8 sheep.

3.5. Discussion

3.5.1 Pharmacokinetics

In the present study, the slow elimination of PBZ has been reported previously in ruminant species. Lees *et al.* (1988) administered PBZ i.v. to cattle at 4.4 mg/kg and obtained a $t_{\frac{1}{2}\beta}$ of 35.9 h, Debacker *et al.* (1980) using 5 mg/kg in cattle obtained a higher value for $t_{\frac{1}{2}\beta}$ of 55.1 h whereas Eberhardson et al. (1979) reported the $t_{\frac{1}{2}\beta}$ values as 43.3 and 42.4 h at dose rates of 3 and 6 mg/kg, respectively. However in the present study, PK values were more like those obtained in goats (Chapter 7). In the goat study, PBZ (4.4 mg/kg i.v.) had a $t_{\frac{1}{2}\beta}$ of 15.43 h, an $AUC_{0-\infty}$ of 1228.84 μg.h/ml, a Cl_B of 4.46 ml/kg/h and a V_{ss} of 87.99 ml/kg.

The plasma disposition of FM following i.v. administration at a single dose rate of 1.1 mg/kg in sheep was best described by a two-compartment model in most of the animals. In fact all of the animal data could be fitted well to a two-compartment model if r was the criterion for the MAICE test since the R^2 values were higher than 0.99 for all animal data fitted. This differs from the previous kinetic study of FM in sheep in our laboratory, where the decline in plasma was best fitted to a three compartment model (Welsh *et al.*, 1993). This may be attributable to the differences of modelling methods. In the previous study, CSTRIP (Sedman & Wagner, 1976) was used for the calculation of estimates. This software program is only useful for curve stripping and the AIC was achieved using the equation derived from curve stripping. In the present study we used PCNONLIN for the kinetic analyses with a weighting factor (1/concentrations) applied to the non-linear estimation. The AIC was calculated from the non-linear regression results. Since the concentration-time curve is non-linear, the model decision and parameter estimation by non-linear methods may be more reliable.

The plasma drug disposition of FM was previously investigated in cattle following i.v. administration and different $t_{\frac{1}{2}\beta}$ values were reported by Landoni *et al.*(1995a), 6.87 h (2.0 mg/kg); Hardee *et al.*(1985), 8.1 h (1.1 mg/kg) and Anderson *et al.* (1990), 3.14 h (1.1 mg/kg). In

the present study the $t_{\frac{1}{2}\beta}$ of 2.48 h (1.1 mg/kg) is shorter than the values [3.81 h (1.0 mg/kg)] in sheep reported previously in our laboratory (Welsh *et al.*, 1993). However the values of Cl_B and V_{ss} between these two studies are almost identical. It is likely that $t_{\frac{1}{2}\beta}$ is a model and algorithm-dependent parameter and that different models or algorithms may generate different values of β. Body clearance and V_{ss} are model independent parameters which are calculated from dose, AUC and MRT.

Distribution into and elimination of FM from tissue-cage exudate and transudate were very slow. The C_{max} in exudate was higher and occurred earlier than in transudate but the elimination from transudate was slower than from exudate. A larger AUC in transudate than in exudate did not reflect greater distribution into the transudate. The concentration-time data indicated that before peak concentration was achieved, exudate FM concentration was higher than transudate FM concentrations at all sampling times. After t_{max} the relative concentrations were reversed. Since a major part of AUC consists of the AUC in the elimination phase, when a comparison of absorption or penetration rate is made, it should be corrected for the elimination rate. Correction using $t_{\frac{1}{2}\beta}$ was described previously (Gibaldi & Perrier, 1982b). However, the number of sampling points for the present drugs in the distribution phase in tissue-cage fluids was insufficient to carry out compartmental modelling while non-compartmental modelling was carried out. Based on statistical moment theory, the MRT indicates the arithmetic average time that each drug molecule remains in the system. Mean residence time is a more reasonable parameter for AUC correction in tissue fluids following non-compartmental modelling than $t_{\frac{1}{2}\beta}$. When the AUC ratios are corrected for MRT, they become slightly higher for exudate to plasma than transudate to plasma. Since the corrected AUC ratios are smaller than the uncorrected, this illustrates that most of the AUC in tissue-cage fluids is composed of the area in the elimination phase. Thus slow elimination plays an important role in drug persistence in the tissue-cage fluids. The distribution for PBZ into exudate and transudate was smaller than for FM. However after the AUC ratios were corrected using the MRT, the ratios were double those of FM. This suggests that the differences in drug elimination rates of PBZ between plasma and tissue-cage fluids were smaller than those of FM. The compartmental modelling also illustrated that the values of elimination half lives of FM in exudate and transudate were much slower than in plasma while similar values were obtained for PBZ in plasma, exudate and transudate.

It was unexpected that the elimination rate from transudate was slower (FM) than or similar (PBZ) to the elimination from exudate and that no significant differences in AUC ratios between exudate to plasma and transudate to plasma were observed in the present study since in common with most of NSAIDs, FM and PBZ have been demonstrated to have slow exudate drug elimination rates and high AUC ratios of exudate to plasma compared with transudate in some animal species, at least in horses (Lees *et al.*, 1986, 1987c) and calves (Landoni *et al.*, 1995a). This suggests a species difference between horses and calves on one hand and sheep on other hand. However similar 'unexpected' results have been observed with tolfenamic acid in dogs using a similar model (McKellar *et al.*, 1994b).

In the present study, both compartmental and non-compartmental modelling were carried out for the analysis of the drug concentration - time data in plasma, exudate and transudate. In the PK analysis for PBZ, compartmental and non-compartmental modelling generated similar parameters for plasma kinetics. However the PK parameters of PBZ in tissue-cage fluids were not consistent between the results analysed using these two methods. For example, the MRT of PBZ in exudate (31.60 ± 1.84 h) and transudate (31.11 ± 2.41 h) using non-compartmental modelling were longer than the elimination half lives analysed by compartmental modelling ($t_{\frac{1}{2}k10}$ was 17.82 ± 1.27 h in exudate and 16.24 ± 1.60 h in transudate). Elimination of PBZ was slow in plasma and tissue-cage fluids. The plasma data were best described by a three compartmental model with rapid bolus i.v. input. However there were insufficient concentration-time points for PBZ in tissue cage fluids to be fitted to a two compartmental model with first order input. Therefore the elimination rates may be underestimated. In this case, the parameters derived using non-compartmental modelling may be more reliable for the comparisons since as discussed previously, non-compartmental modelling generates model-independent parameters.

In the FM-treated group, the results achieved using both approaches were consistent in plasma, exudate and transudate. For example, the MRT of FM in exudate estimated by non-compartmental modelling was 12.98 h and the $t_{\frac{1}{2}k10}$ calculated by compartment modelling was 8.53 h. This agrees with the relationship $t_{\frac{1}{2}\beta} = 0.693 \times \text{MRT}$ (in this case $t_{\frac{1}{2}\beta} = 0.693 \times 12.98 = 8.99$ h).

The extravascular penetration of the drugs in sheep in the present study was extensive as indicated by the high C_{max} in exudate and transudate of 22.32 and 22.07 μg/ml for PBZ, and 1.82 and 1.58 μg/ml for FM, respectively. Following administration of PBZ (4.4 mg/kg, i.v.) to horses, a C_{max} of 12.4 and 6.3 μg/ml in exudate and transudate, respectively was observed (Lees *et al.*, 1986). When FM (1.1 mg/kg) was given i.v. to horses, the exudate C_{max} was about 0.9 μg/ml (Lees *et al.*, 1987c) and in calves FM (2.2 mg/kg) produced a C_{max} of 1.67 μg/ml in exudate and 0.59 μg/ml in transudate although the elimination ($t_{\frac{1}{2}\beta}$=6.87 h) in plasma was slower than the present study (Landoni *et al.*, 1995a). The high extravascular penetration of the drugs in sheep suggests a lower plasma protein drug binding in this species since the unbound drug in the plasma is available to penetrate into both inflamed and non-inflamed tissue-cage fluids while the protein-bound drug can only easily penetrate into the fluids at inflamed sites where the inflammatory reaction results in vasodilatation and high capillary permeability. Previous studies have shown that the percentage protein binding for PBZ was over 97% in the horse (Tobin *et al.*, 1986) but only 60.3% in goats (Boulos *et al.*, 1972). This may explain why there were no significant differences in the drug distribution between exudate and transudate in the present study although further information on protein binding of these drugs in sheep is required. Flunixin has been licensed for use in cows at a dose rate of 2.2 mg/kg in the UK. The present study indicated that half the dose rate in sheep resulted in higher values for AUC and C_{max} in plasma, exudate and transudate than previously reported in cattle (Landoni *et al.*, 1995a). In addition, exudate PGE_2 and skin temperature rise over the inflamed tissue-cages were abolished or significantly inhibited for up to 32 h following i.v. administration of FM at 1.1 mg/kg in sheep. This, therefore, suggests that a dose rate of 1.1 mg/kg (or 1.0 mg/kg) may be sufficient in sheep but this must be supported by toxicity data.

The high distribution into and slow elimination from the tissue-cage fluids is of therapeutic interest since it indicates that these drugs reach extravascular sites (including inflammatory foci) readily and stay there longer than they do in plasma. This supports the explanation for the long therapeutic action of NSAIDs described previously in the horses (Lees *et al.*, 1987c).

3.5.2. Pharmacodynamics

Phenylbutazone produced significant inhibitory effects on COX in equine species (Lees & Higgins, 1986; Lees *et al.*, 1987c; Chapter 6) but its inhibitory effects on COX in sheep in the present study were poor. Similar results have been observed for PBZ in calves (Peter Lees, *et al.*, unpublished observations). Species differences in COX inhibition have also been observed for other NSAIDs. Carprofen did not affect serum TXB_2 or exudate PGE_2 generation in dogs (McKellar *et al.*, 1994a) and horses (Lees *et al.*, 1994) whereas it significantly inhibited those COX products in sheep (Chapter 4). It is unlikely that different distribution of the drug in plasma or in the inflamed tissue cage sites is responsible for these differences because both PBZ and CPF achieved considerably higher concentrations in plasma and exudate in the animal species where the PD effects were less. It is possible that the pharmacological and pathophysiological regulation of COX or the properties of the isoenzymes in mono gastric animals differs from that in ruminants. It is apparent that when the antiinflammatory effects of NSAIDs are determined in animal experiments based on COX inhibition, species differences must be taken into account.

Flunixin meglumine was a potent inhibitor of serum TXB_2 generation in clotting blood and exudate PGE_2 generation in stimulated tissue cages in the present study. It abolished or significantly inhibited both serum TXB_2 and exudate PGE_2 generation for up to 32 h. The inhibitory effect of FM given by i.v. injection on COX was much stronger than that of PBZ although the concentrations of FM in plasma and exudate were much lower than those of PBZ over all sampling times. Previous reports showed that FM effectively inhibited serum TXB_2 and exudate PGE_2 in horses (Lees & Higgins, 1984; Lees *et al.*, 1987a) and calves (Landoni *et al.*, 1995a).

As described in Chapter 1, there are two isoforms of COX which play different roles in physiological and pathological processes. The regulation of these two isoenzymes is pharmacologically distinct (Herschman, 1994; Smith *et al.*, 1994; Johnson *et al.*, 1995). Inhibition of COX-2 is thought to modulate, at least in part, the antipyretic, analgesic and antiinflammatory action of NSAIDs, but the simultaneous inhibition of COX-1 is thought to result in unwanted side effects, particularly those leading to gastric ulceration. The present study

illustrated that the sensitivities of inhibition by PBZ and FM were different in carrageenan-induced tissue COX and PLT COX generated during blood coagulation. The IC_{50} for serum TXB_2 for FM was estimated to be 0.0053 μg/ml occurred 28.96 ±1.86 h after FM administration. Flunixin meglumine produced 50% inhibition for PGE_2 at 144 h when the concentration of FM was estimated to be 0.00019 ± 0.00012 μg/ml in exudate and even lower in plasma. Greater ability against exudate COX than PLT COX may be advantageous since it may confer greater therapeutic effect with a wide safety margin. Phenylbutazone had an IC_{50} of 10.86 ± 1.81 μg/ml against serum TXB_2. It did not produce more than 50 % inhibition on exudate PGE_2 generation at a maximal exudate concentration of 22.32 ± 1.29 μg/ml. A low sensitivity to COX-2 may confer poorer antiinflammatory activity on a NSAID although COX inhibition does not always correlate with PD effect (Lees *et al.,* 1994; McKellar *et al.*, 1994a). This finding confirms previous data which established the IC_{50} of PBZ against COX-1 and COX-2 using human COX-1 and COX cDNA expressed in cultured cells and where the IC_{50} for PBZ was 4.93 μg/ml (16.0 μM) against COX-1 and over 30.84 μg/ml (100 μM) against COX-2 (Laneuville *et al.*, 1994). The high degree of protein binding for nearly all of the NSAIDs implies that only a small fraction of the total drug concentration will be available for inhibition of COX and consequently the true IC_{50} may be much lower. If the protein binding is different in plasma, exudate and cell culture medium, the true IC_{50} value should be adjusted accordingly. A previous study demonstrated that there was no significant difference in total protein between plasma and exudate using the same model as that used in present study but in horses (Lees *et al.*, 1986). However, although differences in protein binding and COX-1 inhibition have been demonstrated between species, protein binding did not account for all the differences in COX-1 inhibition (Galbraith & McKellar, 1996).

The suppressant effects of FM on skin temperature rise over the inflammatory focus was significant while PBZ did not inhibit the temperature increase in the present study. Previous studies in our laboratory using a dog tissue-cage model showed that tolfenamic acid, a NSAID with potent antiinflammatory effects, inhibited exudate PGE_2 and skin temperature simultaneously whereas CPF, another NSAID with no or weak antiinflammatory effects, failed to affect both exudate PGE_2 generation and elevated skin temperature at a dose rate of 4.0 mg/kg (McKellar *et al.*, 1994a, 1994b). It is believed that PGs in exudate mediate an increased blood flow and vasodilatation at acute inflammatory sites and contribute to the temperature increase

associated with inflammation. It is likely that the increased skin temperature over the inflamed cages is a PG-associated symptom in the present model.

Leukotriene B_4 was not inhibited by PBZ or FM, indicating that they do not inhibit 5-LOX *in vivo*. This is not unexpected since PBZ and FM did not inhibit LTB_4 or 12-HETE generation in previous studies (Landoni *et al.*, 1995a). In the present study, LTB_4 increased slightly throughout and significantly at 24 and 48 h following PBZ and FM administration compared with the PLB-administered group. It has been suggested that inhibition of the COX pathway may lead to higher concentrations of LOX derived products by accumulation of substrates and a consequent shift of metabolism (Higgs *et al.*, 1980; Kitchen *et al.*, 1987; Sedgwick *et al.*, 1987).

Both PBZ and FM attenuated the accumulation of WBC in the inflamed sites and a significant decrease in exudate WBC was achieved 12 h after FM treatment ($P<0.05$). This is consistent with the antiinflammatory and antipyretic results in the present study. Previous studies have shown that PBZ and FM did not modify exudate WBC concentration in horses (Lees *et al.*, 1987c). The differences may be due to the effective drug concentrations achieved in the inflamed sites since studies in rats showed that a number of NSAIDs, including PBZ, caused a dose-dependent effect on carrageenan-induced exudate WBC numbers , at high doses NSAIDs (ED_{50} = 52.0 mg/kg) inhibited oedema and WBC accumulation, however at lower doses (0.05 mg/kg) they significantly potentiated WBC migration by 20-70% (Higgs *et al.*, 1980). Although the study in horses (Lees *et al.*, 1987c) and the present study used the same dose rates of PBZ and FM, the C_{max} of both drugs in exudate in the present study were twice as high as reported in horse. This also suggests that the PKs and PDs of NSAIDs differ in animal species so that the data can not be extrapolated from one animal species to another. It is known that LTB_4 is a chemokinetic and chemotactic agent for leukocytes (Ford-Hutchinson *et al.*, 1980). However the present study showed exudate LTB_4 concentrations increased but exudate WBC number decreased in PBZ-treated and FM -treated groups compared with PLB-treated groups. It is possible that the inhibition of vascular permeability or other WBC recruitment factors by the NSAIDs may lead to decreased infiltration and accumulation of leukocytes despite higher LTB_4 concentrations.

3.6. Conclusion

Phenylbutazone and FM produce their antiinflammatory effects by inhibiting COX and thus blocking the formation of COX derived eicosanoids. The inhibitory effects of PBZ and FM were distinct between inducible COX and PLT COX. Flunixin meglumine but not PBZ was a potent COX inhibitor and antiinflammatory drug in sheep *in vivo*. Flunixin meglumine but not PBZ has attractive PK characteristics for clinical use in sheep. Flunixin meglumine readily penetrates into and is slowly eliminated from the inflammatory site so that a dose rate of 1.1 mg/kg i.v. may achieve sufficient drug concentration in plasma and at inflammatory sites to generate therapeutic effects. Tolerance studies are still required before final recommendation on dosage rate can be made.

Chapter 4

Pharmacological effects of carprofen and its enantiomers in sheep

4.1. Introduction

Carprofen (6-chloro-o-methylcarbazole-2-acetic acid) (Fig 4.1) is a NSAID classified as an aryl-propionic acid. It has been licensed for clinical use in horses (0.7 mg/kg) and dogs (2.0-4.0 mg/kg) with indications for inflammation and pain. In animal models of carrageenan-oedema, granuloma-pouch, U.V.-induced erythema and bradykinin and histamine-induced capillary permeability, CPF was shown to possess a greater antiinflammatory potency than PBZ, mefenamic acid and acetyl-salicylic acid (Maeda *et al.*, 1977). It has been demonstrated to decrease significantly the carrageenan-induced oedema in horses (Lees *et al.*, 1994). Studies in cows with endotoxin-induced mastitis showed that rac-CPF treatment (0.7 mg/kg) significantly reduced heart rate and rectal temperature and quarter swelling (Lohuis *et al.*, 1991). It has been demonstrated that CPF was a effective analgesic in dogs (Nolan & Reid, 1993) and sheep (Welsh & Nolan, 1994b, 1995).

The mode of action of CPF is unknown. It has been classified as a weak inhibitor of COX (Strub *et al.*, 1982; Frey, 1992). A previous report that CPF did not substantially modify LT generation in rats indicated that it was not an inhibitor for LOX (Baruth *et al.*, 1985). Previous studies using a subcutaneous tissue cage model of acute inflammation in dogs (McKellar *et al.*, 1994a) and calves (Lees *et al.*, 1996) showed that CPF did not inhibit exudate PGE_2, serum TXB_2 or 12-HETE and this indicates that CPF is inactive against COX isoenzymes (COX-1 and COX-2) and 12-LOX. However, CPF produced moderate suppression of serum TXB_2 and exudate PGE_2 synthesis in horses (Lees *et al.*, 1994) and is a weak inhibitor of PLA_2 (Hope & Welton, 1983). This also indicates a PD difference for CPF between animal species

Carprofen is a chiral compound containing an asymmetrical carbon. There are two enantiomeric forms, the S(+) and R(-) enantiomers. The body is a chiral environment and stereoselectivity exists in a wide range of physiological processes. Therefore each enantiomer may have distinct

pharmacological properties. The CPF preparations currently available for clinical use are racemic mixtures (50:50). Racemic products may be considered as combinations of active drug plus isomeric ballast when one enantiomer has little or no therapeutic effect (Ariens, 1986). It has been shown that like other 2 aryl-propionates, the S(+)CPF enantiomer is consistently more active in antiinflammation but also more toxic than the R(-) isomer in the rats (Gaut *et. al.*, 1975). Previous reports in horses (Graser *et al.*, 1991; Lees *et al.*, 1991a), dogs (McKellar *et al.*, 1994a) and calves (Lees *et. al.*, 1996) showed that R(-)CPF was the predominant enantiomer in plasma, exudate and transudate following administration of rac-CPF.

The purposes of the present study were to investigate the effects of rac-CPF and its enantiomers on COX and 5-LOX derived eicosanoids, inflammatory cells and cutaneous inflammatory heat following i.v. administration of separate rac-CPF, R(-)CPF and S(+)CPF in sheep using a subcutaneous tissue-cage model of inflammation.

4.2. Materials and methods

4.2.1. Animals

Eight male sheep weighing 63 ± 3 kg and aged approximately 1.5 years old at the beginning of first cross-over were used. Hay and water were provided *ad libitum*.

4.2.2. Preparation of injectable solution of R(-) and S(+) CPF

Racemic CPF injectable solution was supplied by Grampian Pharmaceutical Company at a concentration of 50 mg/ml (W/V). The R(-) or S(+) enantiomer was dissolved in excipient solution provided by Grampian Pharmaceutical Company, which contained the same ingredients as the rac-CPF solution but without rac-CPF. The concentration for each enantiomer was 25 mg/ml (W/V).

4.2.2. Experimental protocol

The experiments were carried out on a subcutaneous tissue-cage acute inflammatory model as described in Chapter 2. The cross-over experiment was coded as QM/ZC/03/95 and was started two months after the experiment coded QM/ZC/11/94 (Reported in Chapter 3) using the same tissue-cages and animals.

The study was carried out in a 5 way cross-over Latin square design. An intracaveal injection of carrageenan was given 15 min before administration of the test substances. Each sheep received i.v. 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) and rac-CPF excipient as PLB. Test substances were administered in a single rapid bolus, via the right jugular vein according to the cross-over design (The results of L-NAME treatments were reported in Chapter 4). A four-week washout period was allowed between each occasion of the cross-over. (Table. 4.1.)

Table 4.1. Cross-over design for i.v. administration of drugs and PLB in 8 sheep

	Cross-over occasion				
Sheep No	1	2	3	4	5
143	A	B	C	D	E
144	B	D	E	C	A
145	C	E	A	D	B
146	D	A	B	E	C
147	E	B	C	A	D
148	A	C	D	B	E
149	B	D	E	C	A
150	C	E	A	D	B

All drugs and PLB were administered i.v.. A, rac-CPF (4.0 mg/kg); B, R(-)CPF (2.0 mg/kg); C, S(+)CPF (2.0 mg/kg); D, L-NAME (25 mg/kg) and E, PLB.

The sampling schedule and processing methods were described in Chapter 2. The plasma samples were collected at 20 min prior drug administration (-20 min) and at 5, 15, 30, 45, 60 min and 2, 4, 6, 8, 12, 24, 32, 48, 72, 96, 120 and 144 h following drug administration. Serum samples for determination of TXB_2 were taken at -20 min, 1, 2, 4, 6, 8, 12, 24, 32, 48, 72, 96, 120 and 144 h. Exudate and transudate samples were harvested at -20 min (transudate only), 2, 4, 8, 12, 24, 32, 48, 72, 96, 120 and 144 h. The values for TXB_2, PGE_2, LTB_4, haematology and the temperature record were determined using the methods as described in Chapter 2.

4.2.3. Pharmacodynamic modelling

The data for drug effect on serum TXB_2 versus time were fitted to a Sigmoidal inhibitory effect model using PCNONLIN 4.0 software as described in Chapter 2.

4.2.4. Statistics

The results were expressed as mean ± SE. A five factor ANOVA [treatments of rac-CPF, R(-)CPF, S(+)CPF, L-NAME and PLB] with multiple ways (treatments, sheep, time, periods and sequences) was carried out using GLM routine built in the MINITAB Software package as described in Chapter 2. The effects of ambient temperature and pre-treatment values on the experimental parameters were estimated using an analysis of co-variance routine built in the

GLM model as described in Chapter 2. The differences of the measurements collected at each time point between treatment groups, and between pre-treatment and post-treatment were further confirmed using Fisher's multiple comparisons following ANOVA. The differences between individual animal, time points, cross-over sequences and cross-over periods and their two factor interactions were analysed using ANOVA but without carrying out Fisher's multiple comparisons.

4.3. Results

4.3.1. Effects of rac-CPF, R(-)CPF and S(+)CPF on skin temperature over inflammatory sites

The changes (mean ± SE) in skin temperature between carrageenan-induced and non-treatment tissue-cages in the sheep given PLB, rac-CPF, R(-)CPF or S(+)CPF i.v. are presented in Table 4.2.

Following intracaveal injection of 1 % carrageenan, the skin temperature over the cages increased in a time-related fashion with the development of acute inflammation within the cages. In the PLB-treated group, the mean temperature increase was 0.43 ± 0.09 °C over a 144 h period and a maximal increase of 1.33 ± 0.49 °C was recorded at 12 h. Most of values recorded at each time point were higher than the values recorded 20 min before carrageenan injection (pre-value), however statistical significance ($P<0.05$) was achieved only at 12 h compared with the pre-value due to the large SE (pooled SD = 0.84). Compared with the pre-values, there was a significant increase ($P<0.05$) at 12 h in the R(-)CPF-administered group while no significant difference was observed at any time point in the rac-CPF- or S(+)CPF-treated groups ($P>0.05$). These results indicate an inhibitory effect of rac-CPF and S(+)CPF on inflammatory temperature while R(-)CPF did not modify changes in the temperature.

The analysis illustrated that the changes in skin temperature over the cages were time-related ($P<0.01$). The differences in basal skin temperature over the cages before carrageenan injection (pre-values) had a significant influence on the changes in the temperature following the administration of carrageenan and drugs ($P = 0.01$). The r between the pre-values and post carrageenan administration values was 0.13 ± 0.01 ($P = 0.01$), which indicated that the larger the

pre-values were, the larger the change in the post-values. There was no differences between the sequences of drug administration over the whole cross-over experiment. The changes in temperature were significantly different between the cross-over periods ($P<0.01$) but the ambient temperature did not affect the changes in skin temperature over the cages ($r=-0.01 \pm 0.01$, $P=0.76$).

4.3.2. Effects of rac-CPF, R(-)CPF and S(+)CPF on serum TXB_2 generation

Mean ± SE serum TXB_2 concentrations following i.v. administrations of PLB, rac-CPF, R(-) CPF and S(+)CPF in the sheep are shown in Table 4.3. and the percentage inhibition data are illustrated in Fig 4.2.

There were significant differences between the overall means of the four treatment groups ($P<0.001$). The treatment × time interactions were significantly different ($P<0.001$), indicating that the measurements at each time point differed significantly between the treatment groups. Fisher's multiple comparisons were carried out to confirm the further differences as presented in Table 4.3. Compared with pre-values, PLB treatment did not alter serum TXB_2 concentration at any time point ($P>0.05$), rac-CPF and S(+)CPF treatment decreased serum TXB_2 concentrations significantly for up to 32 h ($P<0.05$) whereas R(-)CPF inhibited serum TXB_2 generation only at 6 and 8 h ($P<0.05$).

Inter-group comparisons showed that there was no significant difference of TXB_2 generation in pre-values between treatment groups ($P>0.05$). Following treatment, the concentrations of TXB_2 in rac-CPF and S(+)CPF were significantly lower than the values in the R(-)CPF and PLB treated group for up to 32 h ($P<0.05$), rac-CPF administration produced higher inhibitory effects than S(+)CPF treatment for up to 6 h ($P<0.05$), and there were no significant differences in serum TXB_2 concentrations between the R(-)CPF and PLB treated groups for up to 32 h ($P>0.05$) but thereafter TXB_2 concentrations in the R(-)CPF administered group were higher than in PLB-administered group ($P<0.05$).

Using PD modelling, the data for percentage TXB_2 inhibition against time was fitted to a sigmoidal inhibitory effect model with a Hill constant and the results are given in Table 4.4.

Racemic CPF administration produced an E_{max} of 78.84 ± 3.19 % and an IT_{50} of 19.00 ± 3.20 h. The treatment of S(+)CPF generated an E_{max} of 68.10 ± 6.15% and an IT_{50} of 24.40 ± 4.89 h. The accumulative inhibitory effect, indicated by the area under percentage inhibition-time curve in a period of 1-72 h, was 1663.50 ± 135.98 for rac-CPF and 1733.70 ± 334.93 for S(+)CPF and this indicates a similar inhibitory potency of these drug on serum TXB_2 generation (P>0.05). There were no significant differences for any parameters between these two treatment group (P>0.05).

The analysis also illustrated that the differences in serum TXB_2 inhibition were significant in treatment sequence and cross-over period (P<0.001). Analysis of co-variance demonstrated that the basal serum TXB_2 generation during blood clotting (pre-treatment values) had a significant effect on TXB_2 inhibition (P<0.01) and a coefficient of -0.33 ± 0.01 (P<0.01) indicated that the higher the pre-values were, the lower inhibitory effect the drug produced.

4.3.3. Effects of rac-CPF, R(-)CPF and S(+)CPF on exudate PGE_2 generation

Table 4.5. and Fig. 4.3. demonstrate the changes in PGE_2 generation in carrageenan-induced inflammatory exudate following i.v. administrations of PLB, rac-CPF, R(-)CPF and S(+)CPF.

Before carrageenan injection into the cages the tissue-cage fluid was transudate in which PGE_2 was undetectable (below the analytic limit of quantification). Intracaveal injection of 1 % carrageenan induced the generation of a large quantity of PGE_2 in the inflammatory exudate in a time-related fashion. The increase in exudate PGE_2 generation was significant from 4 to 32 h (P<0.05) and a peak concentration of 86.24 ± 11.75 ng/ml was achieved at 12 h which was 4 h after the second injection of carrageenan into the cages.

The differences of exudate PGE_2 concentrations between the overall means of PLB (35.35 ± 4.64 ng/ml), rac-CPF (8.32 ± 2.28 ng/ml), R(-)CPF (36.87 ± 4.67 ng/ml) and S(+)CPF (11.78 ± 2.88 ng/ml) treatments were significant (P<0.001). Fisher's comparisons showed that the values in the rac-CPF and S(+)CPF treated groups were significantly different from those in the PLB and R(-)CPF treated groups (P<0.001) and there were no significant differences between rac-CPF and S(+)CPF groups or between PLB and R(-)CPF groups (P>0.05).

The measurements at each time point between the PLB, rac-CPF, R(-)CPF and S(+)CPF treated groups (treatment × time interactions) were significantly different ($P<0.001$). When the post-values were compared with the pre-values, significant increase ($P<0.05$) in PGE_2 concentrations was observed from 2 to 32 h in the PLB-treated and R(-) CPF-treated groups, only at 12 h in the rac-CPF-treated group, and at 12 and 24 h in the S(+)CPF-treated group.

Inter-group comparisons showed that the values at 2 h were not significantly different between all treatment groups ($P>0.05$), exudate PGE_2 concentrations were not significantly different ($P>0.05$) between the rac-CPF-treated and S(+)CPF treated groups or between the PLB treated and R(-)CPF treated groups at any time point over a period of 144 h. The concentrations of exudate PGE_2 in the rac-CPF treated and S(+)CPF treated groups were significantly lower than the values in the PLB treated and R(-)CPF treated group from 4 to 32 h ($P<0.05$).

Analysis of variance showed that the response of exudate PGE_2 production to carrageenan induction was significantly different between individual sheep ($P<0.001$) and this exerted a significant effect on exudate COX inhibition produced by the drug treatments ($r = -0.41 \pm 0.01$, $P<0.001$). Again this indicates a competitive inhibitory dynamics.

The analysis also showed that there were significant differences ($P<0.001$) in exudate PGE_2 generation in treatment sequences, experimental periods and time × sheep interactions. This indicates that a number of factors influenced the experiment.

4.3.4. Effects of rac-CPF, R(-)CPF and S(+)CPF on exudate LTB_4 generation

Mean ± SE concentrations of LTB_4 in carrageenan-induced inflammatory exudate following i.v. administrations of PLB, rac-CPF, R(-)CPF and S(+)CPF are presented in Table 4.6.

Leukotriene B_4 was undetectable (below the analytical limit of quantification) in the transudate before injection of carrageenan into the cages. Following intracaveal injection of carrageenan, exudate was generated and LTB_4 production increased steadily in a time-related fashion with a peak concentration of 1.83 ± 0.44 ng/ml at 12 h.

Analysis of variance showed that there were significant differences ($P<0.001$) in the overall mean concentrations of exudate LTB_4 between the PLB (1.07 ± 0.07 ng/ml), rac-CPF (1.20 ± 0.06), R(-)CPF (1.06 ± 0.08 ng/ml) and S(+)CPF (1.72 ± 0.18 ng/ml) groups, however further testing using Fisher's multiple comparisons indicated that only the S(+)CPF group significantly differed from the other three groups ($P<0.01$). Compared with the pre-values, there was a significant increase ($P<0.05$) in LTB_4 concentrations from 4 to 144 h in the rac-CPF, R(-)CPF and S(+)CPF groups and from 8 to 120 h in the PLB group. Compared with the PLB group, the values in rac-CPF, R(-)CPF and S(+)CPF groups were higher at most of the time points but statistical significance was achieved only at 12, 48 and 72 h in the S(+)CPF group. Exudate LTB_4 concentrations in the S(+)CPF group were also higher than all other groups at 12, 48 and 72 h ($P<0.05$). The concentrations of exudate LTB_4 were not significantly different between the PLB, rac-CPF and R(-)CPF groups at any time point ($P>0.05$). There were no significant differences ($P>0.05$) in LTB_4 generation with experimental sequences and periods. The response of LTB_4 generation to carrageenan induction was significantly different between individual sheep ($P<0.001$) but this did not alter the effects of the treatments ($r = 0.16$, $P>0.05$).

4.3.5. Effects of rac-CPF, R(-)CPF and S(+)CPF on leukocyte recruitment at inflammatory sites

The changes in WBC numbers ($\times 10^9$ cells/L) in carrageenan-induced inflammatory exudate following i.v. administrations of PLB, rac-CPF, R(-)CPF and S(+)CPF are illustrated in Fig 4.4.

Intracaveal injection of 1 % carrageenan caused a time-related recruitment of WBC in the inflammatory exudate. The WBC numbers increased slowly following the first injection of carrageenan ($5.35 \pm 1.02 \times 10^9$ cells/L at 4 h and $5.49 \pm 1.32 \times 10^9$ cells/L at 8 h) and steeply following the second injection. A plateau was reached in 12-24 h (17.63 ± 3.72-$17.03 \pm 4.44 \times 10^9$ cells /L) before the rapid drop at 48 h ($5.35 \pm 0.80 \times 10^9$ cells/L).

There was no significant difference ($P>0.05$) for WBC accumulation in exudate between the overall means of the PLB ($10.17 \pm 1.48 \times 10^9$ cells/L), rac-CPF (11.50 ± 1.92 cells/L), R(-)CPF (11.85 ± 1.58 cells/L) and S(+)CPF (11.69 ± 1.37 cells/L) groups. Compared with the PLB-treated group for the WBC values at each time point, i.v. administration of rac-CPF, R(-)CPF and S(+)CPF led to a slight decrease at 12 h but this was followed an moderate increase at 24 h

at which time a significant difference was observed between the rac-CPF and PLB treated group ($P<0.05$).

The analysis also showed that there were significant differences in exudate WBC numbers in experimental sequences and periods ($P<0.05$). The WBC numbers in carrageenan-induced exudate were significantly different between individual animals ($P<0.01$) but this did not alter the effects of treatments ($r=0.014 \pm 0.017$, $P = 0.892$).

4.3.6. Effects of rac-CPF, R(-)CPF and S(+)CPF on leukocyte and PLT numbers in blood

Intravenous administrations of rac-CPF, R(-)CPF and S(+)CPF did not modify the numbers of leukocytes and PLT significantly in the venous blood at any time point ($P>0.05$) (Table 4.7, 4.8).

Table 4.2. The changes in skin temperature over the cages following i.v. administration of PLB, rac-CPF (4.0 mg/kg), R(-)CPF (2.0 mg/kg) and S(+)CPF (2.0 mg/kg) in sheep

Time	PLB	rac-CPF	R(-)CPF	S(+)CPF
Pre-values	0.08 ± 0.21	0.03 ± 0.19	-0.12 ± 0.34	0.39 ± 0.32
1	0.29 ± 0.37	-0.30 ± 0.33	0.09 ± 0.50	-0.12 ± 0.17
2	0.29 ± 0.27	0.41 ± 0.44	0.65 ± 0.19	0.68 ± 0.26
4	0.25 ± 0.43	0.55 ± 0.21	0.46 ± 0.46	0.79 ± 0.25
6	0.72 ± 0.18	0.61 ± 0.36	0.70 ± 0.21	0.59 ± 0.26
8	0.74 ± 0.18	0.55 ± 0.30	0.58 ± 0.21	0.13 ± 0.31
12	1.33 ± 0.49*	0.70 ± 0.46	0.86 ± 0.25*	0.85 ± 0.28
24	0.43 ± 0.26	0.12 ± 0.44	0.01 ± 0.33	0.28 ± 0.33
32	0.29 ± 0.26	0.54 ± 0.38	0.25 ± 0.28	0.56 ± 0.32
48	0.16 ± 0.26	0.80 ± 0.26	0.45 ± 0.33	0.55 ± 0.44
72	0.24 ± 0.30	-0.06 ± 0.41	-0.06 ± 0.24	0.14 ± 0.27
96	0.05 ± 0.41	0.33 ± 0.26	0.38 ± 0.27	0.49 ± 0.14
120	0.29 ± 0.29	0.15 ± 0.34	0.43 ± 0.25	-0.08 ± 0.30
144	0.49 ± 0.31	0.20 ± 0.31	0.70 ± 0.49	0.86 ± 0.20

The values (°C) are the difference of skin temperature between the cages into which carrageenan was injected and the cages into which carrageenan was not injected and expressed in mean ± SE in 8 sheep. (*) $P<0.05$ compared with the measurement prior to carrageenan injection into the cages.

Table 4.3. Concentrations of serum TXB_2 following i.v. administration of PLB, rac-CPF (4.0 mg/kg), R(-)CPF (2.0 mg/kg) and S(+)CPF (2.0 mg/kg) in sheep.

Time (h)	PLB[P]	rac-CPF[C]	R(-)CPF[R]	S(+)CPF[S]	Group comparisons
Pre-values	27.13 ± 1.86	29.45 ± 1.96	29.75 ± 2.67	30.61 ± 1.21	(P,C,R,S)
1	24.99 ± 2.48	5.71 ± 1.16*	27.20 ± 2.53	9.98 ± 3.06*	(C)(S)(R,P)
2	26.36 ± 2.68	7.07 ± 1.21*	28.72 ± 1.84	11.12 ± 2.43*	(C)(S)(R,P)
4	26.00 ± 2.25	7.83 ± 1.51*	28.00 ± 3.24	13.21 ± 3.28*	(C)(S)(R,P)
6	25.72 ± 2.19	10.69 ± 3.81*	24.17 ± 1.92*	14.20 ± 3.49*	(C)(S)(R,P)
8	27.89 ± 2.29	14.50 ± 1.56*	26.30 ± 2.76*	13.64 ± 2.66*	(C,S)(R,P)
12	25.61 ± 2.16	12.57 ± 1.81*	26.88 ± 3.53	13.89 ± 1.99*	(C,S)(R,P)
24	26.71 ± 2.22	21.45 ± 1.87*	28.85 ± 2.01	20.95 ± 2.76*	(C,S)(R,P)
32	26.36 ± 2.59	23.14 ± 3.46*	29.19 ± 2.29	20.35 ± 3.37*	(C,S)(R,P)
48	24.92 ± 2.59	27.02 ± 1.60	29.12 ± 2.62	28.85 ± 2.22	(C,R,S)(C,P,S)
72	27.09 ± 1.66	29.39 ± 1.88	30.93 ± 1.98	29.73 ± 2.07	(C,R,S)(C,P,S)

The values (ng/ml) are expressed as mean ± SE in 8 sheep. (*) $P<0.05$ compared with the measurement prior to treatments. Groups in the different brackets are significantly different ($P<0.05$).

Table 4.4 Pharmacodynamic parameters of TXB_2 inhibition following i.v. administrations of PLB, rac-CPF (4.0 mg/kg), R(-)CPF (2.0 mg/kg) and S(+)CPF (2.0 mg/kg) in sheep (n=8, mean ± SE)

Parameters	PLB	rac-CPF	R(-)CPF	S(+)CPF
E_{max} (%)	8.71 ± 4.74	78.84 ± 3.19	7.23 ± 6.82	68.10 ± 6.15
IT_{50} (h)	N/A	19.00 ± 3.20	N/A	24.40 ± 4.89
γ	N/A	2.49 ± 0.81	N/A	4.77 ± 2.06
$AUC_{0\text{-}last}$	N/A	1663.50 ± 135.98	N/A	1733.70 ± 334.93

The data of percentage serum TXB_2 inhibition against time was fitted to a Sigmoidal inhibitory effect model as described in Chapter 2. E_{max} maximal percentage inhibition. IT_{50} = time at which the drug produces 50% of E_{max}. γ = Hill constant. AUC = area under the curve using percentage TXB_2 inhibition against time plot. N/A = not applicable.

Table 4.5. Concentrations of exudate PGE_2 following i.v. administration of PLB, rac-CPF (4.0 mg/kg), R(-)CPF (2.0 mg/kg) and S(+)CPF (2.0 mg/kg) in sheep.

Time (h)	PLB (P)	Rac-CPF (C)	R(-)CPF (R)	S(+)CPF (S)	Group comparisons
Pre-values	UD	UD	UD	UD	(P,C,R,S)
2	7.72 ± 6.81	0.34 ± 0.34	2.38 ± 1.39	0.00 ± 0.00	(P,C,R,S)
4	41.19 ± 13.51*	6.08 ± 5.89	43.41 ± 13.41*	15.01 ± 12.00	(C,S)(R,P)
8	79.78 ± 11.18*	18.01 ± 10.05	86.28 ± 12.31*	19.58 ± 13.68	(C,S)(R,P)
12	86.24 ± 11.75*	38.38 ± 14.22*	101.67 ± 7.88*	39.67 ± 14.54*	(C,S)(P,R)
24	62.45 ± 16.07*	11.00 ± 6.10	67.62 ± 13.24*	21.91 ± 9.56*	(C,S)(R,P)
32	38.27 ± 9.78*	8.81 ± 3.93	49.88 ± 15.57*	19.79 ± 7.71	(C,S)(R,P)(S,P)
48	7.99 ± 3.25	0.66 ± 0.32	20.20 ± 8.57	0.97 ± 0.48	(P,C,S,R)
72	6.50 ± 3.59	0.00 ± 0.00	7.40 ± 4.81	0.22 ± 0.22	(P,C,S,R)
96	2.32 ± 1.02	0.00 ± 0.00	9.44 ± 6.73	0.84 ± 0.52	(P,C,S,R)
120	18.39 ± 18.39	0.35 ± 0.35	0.74 ± 0.45	1.29 ± 0.89	(P,C,S,R)
144	4.54 ± 4.04	0.15 ± 0.15	9.34 ± 7.20	0.35 ± 0.35	(P,C,S,R)

The values (ng/ml) are expressed in mean ± SE in 8 sheep. (*) $P<0.05$ compared with the measurement prior to treatments. Groups in the different brackets are significantly different ($P<0.05$). UD, undetectable.

Table 4.6. Concentrations of exudate LTB_4 following i.v. administration of PLB, rac-CPF (4.0 mg/kg), R(-)CPF (2.0 mg/kg) and S(+)CPF (2.0 mg/kg) in sheep.

Time (h)	PLB	rac-CPF	R(-)CPF	S(+)CPF
Pre-values	U/D	U/D	U/D	U/D
2	0.41 ± 0.09	0.78 ± 0.15	0.52 ± 0.08	0.52 ± 0.12
4	0.85 ± 0.10	0.89 ± 0.11*	0.84 ± 0.19	1.14 ± 0.19*
8	1.28 ± 0.19*	1.05 ± 0.05*	1.00 ± 0.23*	1.74 ± 0.31*
12	1.83 ± 0.44*	1.88 ± 0.34*	1.35 ± 0.31*	2.85 ± 0.87*#
24	1.06 ± 0.15*	1.09 ± 0.18*	0.97 ± 0.18*	1.31 ± 0.17*
32	1.21 ± 0.15*	1.42 ± 0.22*	1.24 ± 0.23*	1.56 ± 0.30*
48	1.33 ± 0.19*	1.26 ± 0.15*	1.12 ± 0.25*	2.71 ± 1.02*#
72	1.07 ± 0.24*	1.25 ± 0.11*	0.89 ± 0.15*	2.70 ± 0.76*#
96	0.90 ± 0.24*	1.06 ± 0.15*	1.14 ± 0.22*	1.59 ± 0.28*
120	0.96 ± 0.16*	1.03 ± 0.19*	1.48 ± 0.66*	1.39 ± 0.51*
144	0.83 ± 0.09	1.26 ± 0.15*	1.21 ± 0.25*	1.03 ± 0.26*

The values (ng/ml) are expressed in mean ± SE in 8 sheep. UD, undetectable. (*) $P<0.05$, compared with the measurements prior to carrageenan injection and (#) $P<0.05$, compared with the animals treated with PLB, CPF and R(-)CPF.

Table 4.7. Numbers of leukocytes in venous blood following i.v. administration of PLB, rac-CPF (4.0 mg/kg), R(-)CPF (2.0 mg/kg) and S(+)CPF (2.0 mg/kg) in sheep.

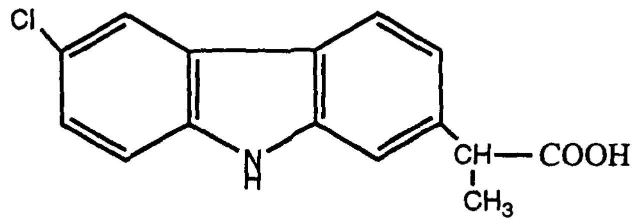
Time (h)	PLB	rac-CPF	R(-)CPF	S(+)CPF
Pre	7.68 ± 0.69	7.84 ± 0.47	7.24 ± 0.52	7.58 ± 0.67
4	7.57 ± 0.66	7.33 ± 0.51	7.27 ± 0.73	7.42 ± 0.88
24	7.68 ± 0.58	7.33 ± 0.48	6.95 ± 0.54	6.61 ± 0.82
48	6.84 ± 0.59	7.14 ± 0.45	7.25 ± 0.57	6.45 ± 0.48

The values ($\times 10^9$ cells/L) are expressed in mean ± SE in 8 sheep.

Table 4.8. Numbers of PLT in venous blood following i.v. administration of PLB, rac-CPF (4.0 mg/kg), R(-)CPF (2.0 mg/kg) and S(+)CPF (2.0 mg/kg) in sheep.

Time (h)	PLB	rac-CPF	R(-)CPF	S(+)CPF
Pre-values	179.13 ± 16.77	192.88 ± 18.93	180.38 ± 16.72	197.50 ± 18.97
4	195.29 ± 18.65	168.57 ± 19.58	161.83 ± 19.81	189.83 ± 13.67
24	193.75 ± 22.69	188.75 ± 13.68	188.88 ± 22.48	194.00 ± 15.96
48	201.25 ± 18.87	212.88 ± 16.37	196.63 ± 19.25	208.38 ± 19.51

The values ($\times 10^9$ cells/L) are expressed in mean ± SE in 8 sheep.



Carprofen

Fig. 4.1. Chemical structure of carprofen

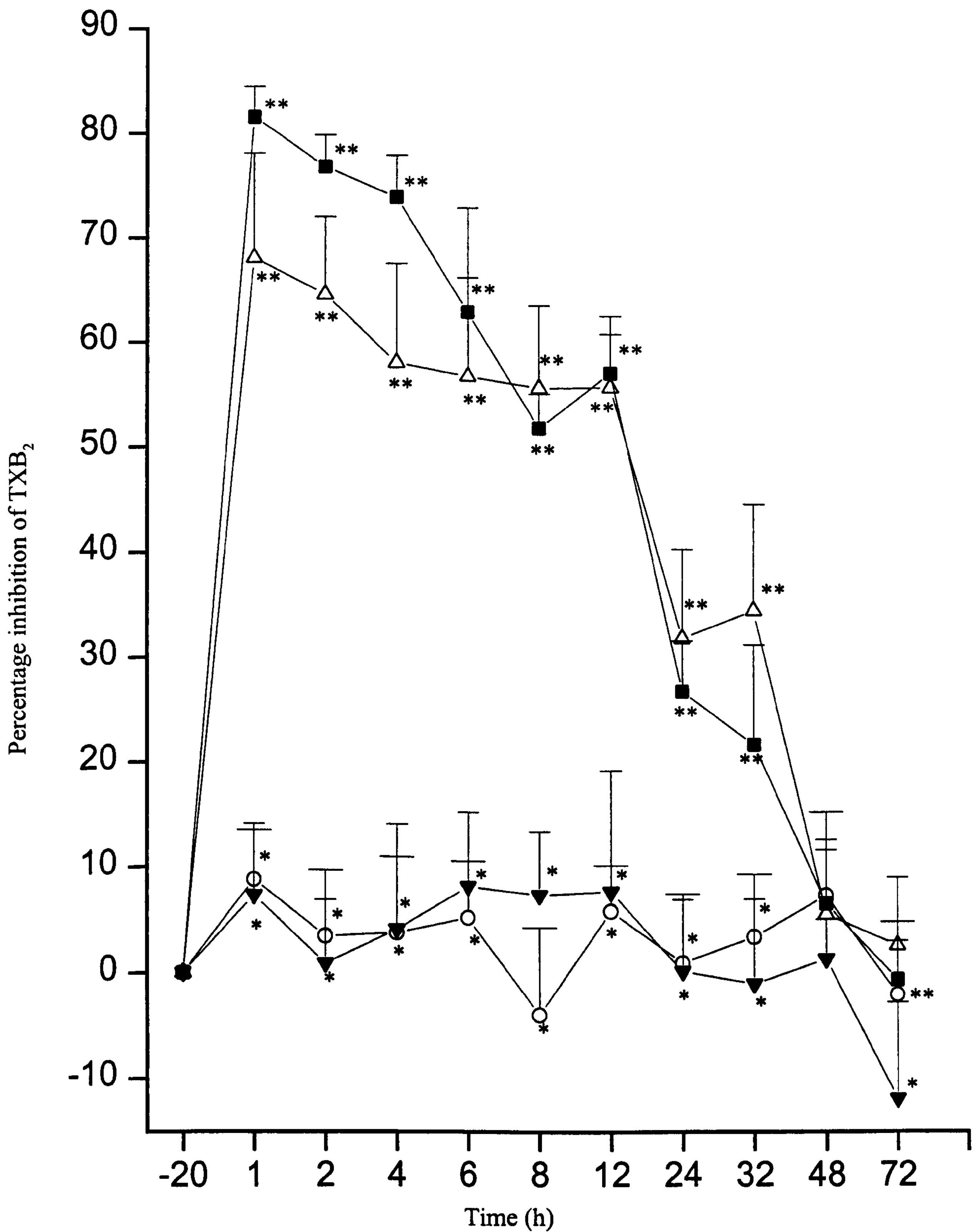


Fig. 4.2. Percentage inhibition of serum TXB_2 following i.v. administrations of CPF (—■—, 4.0 mg/kg), R(-)CPF (—▼—, 2.0 mg/kg), S(+)CPF (—△—, 2.0 mg/kg) and PLB (—○—) in sheep (mean ± SE, n=8). The data points at each time with different numbers of asterisks are significantly different (P<0.05).

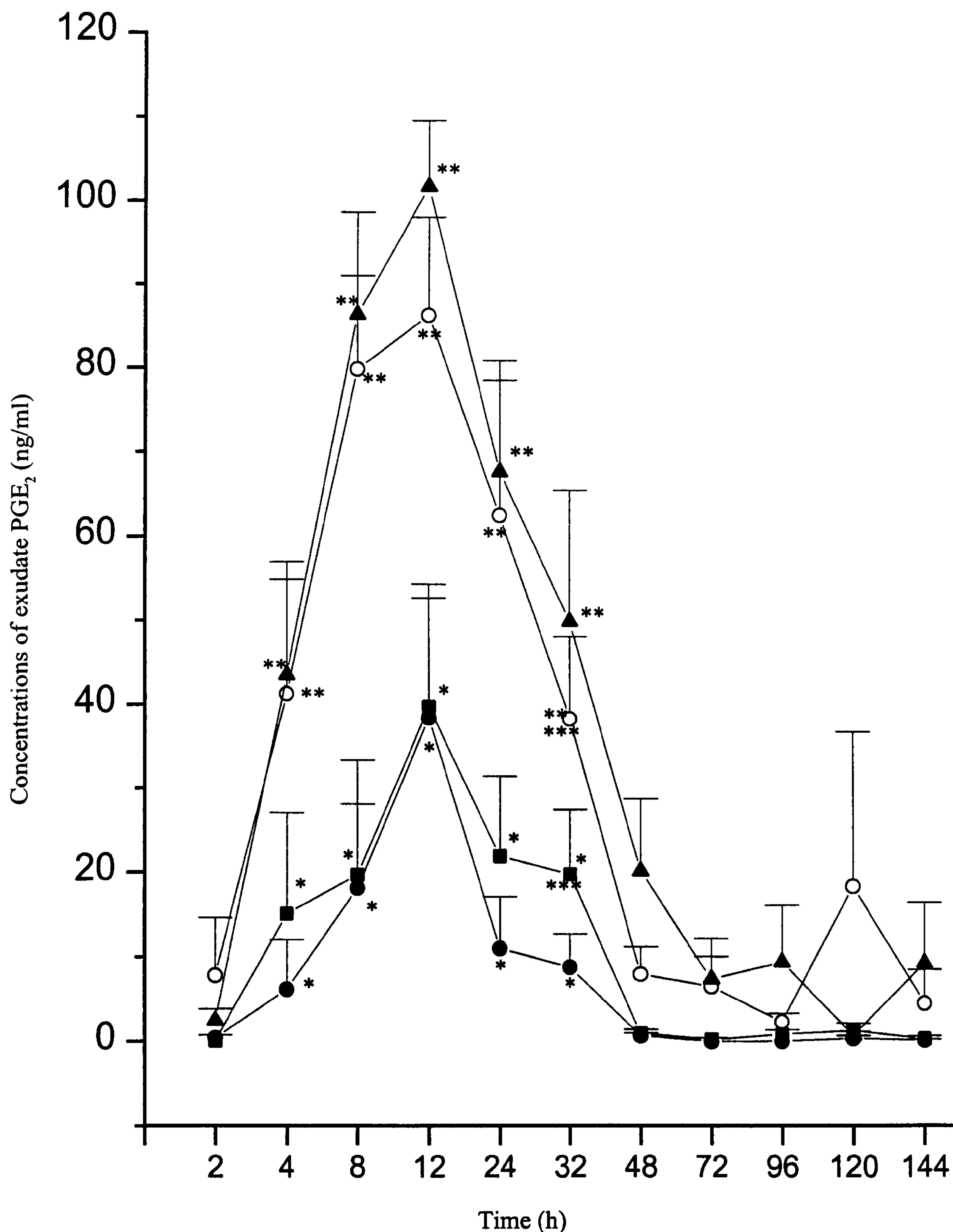


Fig. 4.3. Concentrations versus times plot of PGE_2 following i.v. administration of PLB (—○—), CPF (—●—, 4.0 mg/kg), R(-)CPF (—▲—, 2.0 mg/kg) and S(+)CPF (—■—, 2.0 mg/kg) in sheep (n=8, mean ± SE). The data points at each time with different numbers of asterisks are significantly different ($P<0.05$).

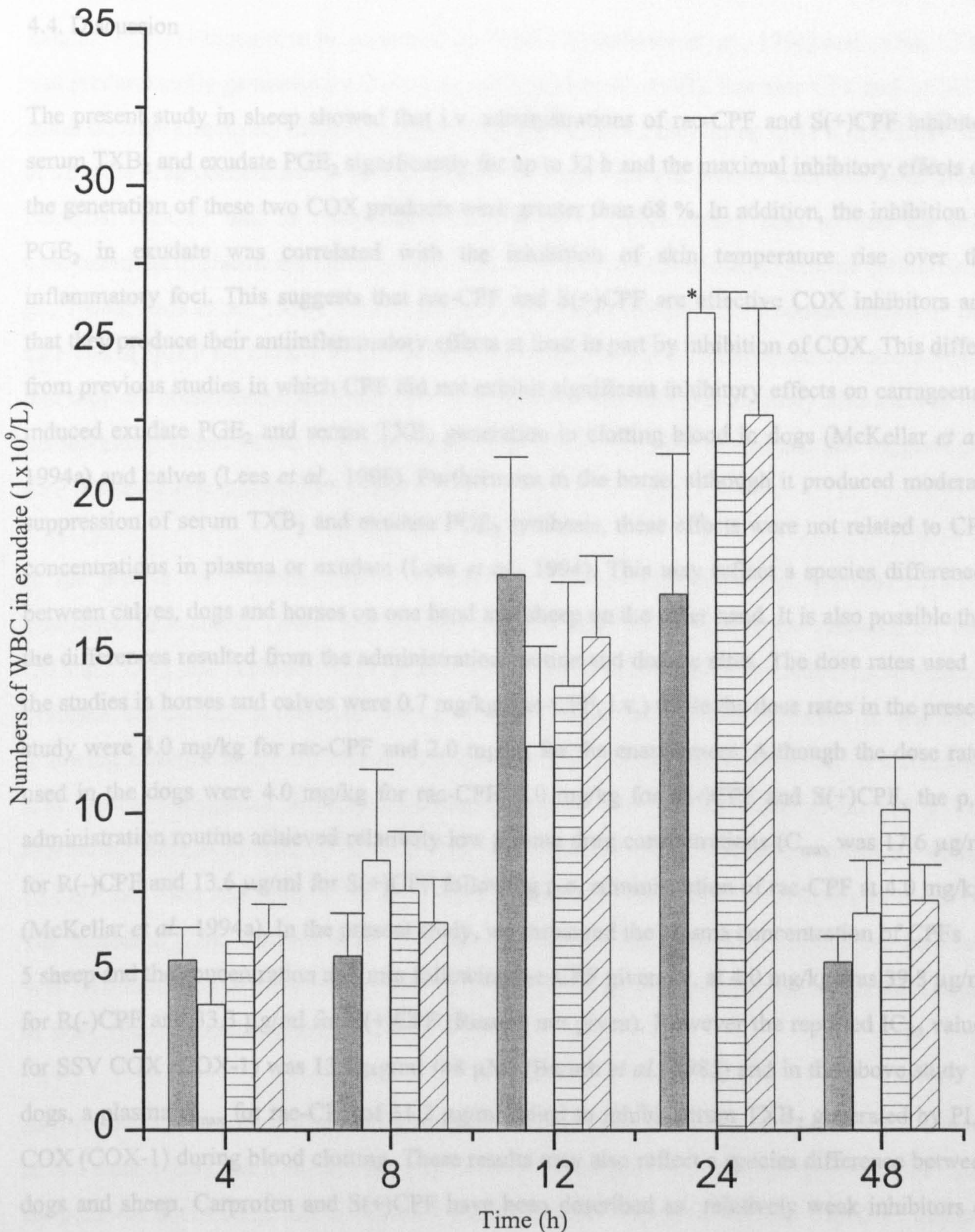


Fig. 4.4. Number ($1x10^9$/L) versus time plot of WBC in exudate following i.v. adminitrations of PLB ( ), CPF (, 4.0 mg/kg), R(-)CPF (, 2.0 mg/kg) and S(+)CPF (, 2.0 mg/kg) in sheep (N=8, mean ± SE). * P<0.05, compared with PLB-treated group.

4.4. Discussion

The present study in sheep showed that i.v. administrations of rac-CPF and S(+)CPF inhibited serum TXB_2 and exudate PGE_2 significantly for up to 32 h and the maximal inhibitory effects on the generation of these two COX products were greater than 68 %. In addition, the inhibition of PGE_2 in exudate was correlated with the inhibition of skin temperature rise over the inflammatory foci. This suggests that rac-CPF and S(+)CPF are effective COX inhibitors and that they produce their antiinflammatory effects at least in part by inhibition of COX. This differs from previous studies in which CPF did not exhibit significant inhibitory effects on carrageenan induced exudate PGE_2 and serum TXB_2 generation in clotting blood in dogs (McKellar *et al.*, 1994a) and calves (Lees *et al.*, 1996). Furthermore in the horse, although it produced moderate suppression of serum TXB_2 and exudate PGE_2 synthesis, these effects were not related to CPF concentrations in plasma or exudate (Lees *et al.*, 1994). This may reflect a species differences between calves, dogs and horses on one hand and sheep on the other hand. It is also possible that the differences resulted from the administration routine and dosage rates. The dose rates used in the studies in horses and calves were 0.7 mg/kg (rac-CPF, i.v.) while the dose rates in the present study were 4.0 mg/kg for rac-CPF and 2.0 mg/kg for the enantiomers. Although the dose rates used in the dogs were 4.0 mg/kg for rac-CPF, 2.0 mg/kg for R(-)CPF and S(+)CPF, the p.o. administration routine achieved relatively low plasma drug concentrations (C_{max} was 17.6 μg/ml for R(-)CPF and 13.6 μg/ml for S(+)CPF following p.o. administration of rac-CPF at 4.0 mg/kg) (McKellar *et al.,* 1994a). In the present study, we measured the plasma concentration of CPFs in 5 sheep and the concentration at 5 min following rac-CPF given i.v. at 4.0 mg/kg was 39.8 μg/ml for R(-)CPF and 33.3 μg/ml for S(+)CPF (Results not given). However the reported IC_{50} values for SSV COX (COX-1) was 13.1 μg/ml (48 μM) (Baruth *et al.*, 1985) and in the above study in dogs, a plasma C_{max} for rac-CPF of 31.2 μg/ml failed to inhibit serum TXB_2 generated by PLT COX (COX-1) during blood clotting. These results may also reflect a species difference between dogs and sheep. Carprofen and S(+)CPF have been described as relatively weak inhibitors of COX in several standard animal models in rats and mice (Baruth *et al.*, 1985). The IC_{50} of rac-CPF for the COX from SSV was 48 μM while the IC_{50} for indomethacin was 0.5 μM. It is also about 100 times less potent at inhibiting COX activity in inflammatory cells (in this study, human synovial cells and rat peritoneal PMN) than indomethacin (Baruth *et al.*, 1985).

Exudate PGE_2 is thought to be generated by COX-2 (Masferrer *et. al.*, 1994) and serum TXB_2 was predominantly generated by COX-1 in PLT (Funk *et al.*, 1991). Racemic CPF and S(+)CPF administration effectively inhibited serum TXB_2 and exudate PGE_2 for up to 32 h. It is likely that at 32 h the drug concentrations in exudate may be higher than in plasma since like other acidic NSAIDs, rac-CPF and its enantiomers accumulate in inflammatory sites so that the drug concentrations in plasma are initially higher and subsequently lower than in exudate (McKellar *et al.*, 1994a; Lees *et al.*, 1996). This suggests that the effective inhibitory concentrations of rac-CPF and S(+)CPF for COX-2 would be the same or higher than for COX-1 and this also suggests that they are non-selective inhibitors for COX isoenzymes. The present study also showed that the inhibitory effects of rac-CPF and S(+)CPF on COX were time related and this suggests that CPF is a reversible inhibitor for COX.

In the present study administration of rac-CPF and S(+)CPF caused an increase in LTB_4 generation in exudate. This is not unexpected since arachidonic acid is the substrate for both COX and LOX and inhibition of COX may lead to the accumulation of arachidonic acid which is available for 5-LOX to catalyse into LTB_4. The same results were observed in the sheep following administration of FM and PBZ (Chapter 3). The metabolic diversion of arachidonic acid has been suggested in previous reports (Higgs *et al.*, 1980; Kitchen *et al.*, 1985). This illustrates that rac-CPF and S(+)CPF are not inhibitors of 5-LOX. The inactivity of CPF against 12-LOX has been also reported in dogs (McKellar *et al.*, 1994a) and calves (Lees *et al.*, 1996). The increased LTB_4 concentration in exudate in the present study may explain the increased WBC accumulation in the exudate since LTB_4 is a powerful chemokinetic and chemotactic agent to WBC (Ford-Hutchinson *et al.*, 1980).

Aryl-propionic NSAIDs, including CPF, contain an asymmetric carbon atom and therefore exists as two enantiomers. Each enantiomer may have differing pharmacological properties in the chiral environment in the body. The enantiomer that has greater biological effect is called the eutomer and the enantiomer with lesser biological effect is the distomer. Significant differences in potency and biological effects between these enantiomeric pairs have been reported. It was believed that the S(+)-isomer is more potent than R(-)-isomer in producing therapeutic and side effects. This has, at least, been shown for CPF in rats and mice (Baruth *et al.*, 1985) and KPF in

several experimental animal species and man (Harris & Vavan, 1985) and calves (Landoni *et al.*, 1995b). The antiinflammatory differences between the enantiomeric pair of rac-CPF were not previously demonstrated in domestic animal species since rac-CPF and its enantiomers did not show any significant effects on the tested inflammatory mediators or symptoms (McKellar *et al.*, 1994a; Lees *et al.*, 1994; Lees *et. al.*, 1996).

The present study confirmed that the antiinflammatory effects of the enantiomers of CPF were different and that the S(+)CPF was the eutomer and the R(-)CPF the distomer in sheep. Racemic CPF contains 50 % of each isomer so that half dose rates of separate isomers are equal to a normal dose of rac-CPF for the comparison of enantiomers. Racemic-CPF was shown to have an equivalent potency with S(+)CPF in inhibiting inflammatory temperature rise over the cages whereas R(-)CPF administration did not affect the temperature. The difference of serum TXB_2 inhibition between rac-CPF and S(+)CPF was very small although significance was achieved for up to 6 h ($P<0.05$). The administration of R(-)CPF significantly inhibited serum TXB_2 generation at 6 and 8 h ($P<0.05$) although the maximal percentage inhibition was only about 8 %. This suggests that R(-)CPF contributes inhibitory effects for serum TXB_2 generation following the administration of rac-CPF. Administration of R(-)CPF not only failed to inhibit exudate PGE_2 generation but also slightly upregulated PGE_2 generation in exudate at most of the time points although no significant difference was achieved. It is believed that inhibition of COX-2 modulates the therapeutic action of NSAIDs (the antipyretic, analgesic and antiinflammatory effects) and the simultaneous inhibition of COX-1 lead to unwanted side effects, particularly those causing gastric ulceration and renal papillary necrosis. As mentioned above serum TXB_2 generation is associated with COX-1 and exudate PGE_2 is produced predominantly by COX-2. It is, therefore, likely that following administration of rac-CPF, R(-)CPF does not contribute to the therapeutic effects in inflammation but may contribute to some side effects, S(+)CPF is conferred with the therapeutic effects and may also predominate in the production of side effects.

Previous studies showed that CPF was an inhibitor for PLA_2 (Hope & Welton, 1983) and was more potent than the other NSAIDs as an inhibitor of arachidonic acid release from cellular lipids (Georgiadis *et al.*, 1983). However the present study failed to support these findings. The action of PLA_2 is to cause the release of arachidonic acid which can be catalysed into PGs via the COX path way or into LTB_4 via LOX path way. In the present study administration of rac-CPF

and its active enantiomer, S(+)CPF, lead to an increased LTB_4 concentration in exudate. Although this was considered to be a result of substrate diversion associated with arachidonic acid metabolism, concentrations of LTB_4 would be expected to decrease if PLA_2 were effectively inhibited.

In conclusion CPF is an effective COX inhibitor in sheep, CPF produces antiinflammatory effects which are predominately attributed to the inhibitory activity of S(+)CPF on COX isoenzymes while the R(-) enantiomer had no therapeutic effects but may be associated with some of the side effects of rac-CPF.

Chapter 5

The roles of nitric oxide and its interactions with eicosanoid generation in acute inflammation in sheep

5.1. Introduction

Nitric oxide is generated during the metabolism of L-arginine by the NADPH-dependent enzyme, NOS. A role for NO has been demonstrated in acute inflammation. In mice, NO was associated with inflammatory pain and inhibition of NO by L-NAME exhibited antinociceptive activity (Moore *et al.*, 1991). In rats, NO was implicated in the acute inflammatory response following footpad injection of carrageenan (Lalenti *et al.*, 1992) or the topical application of mustard (Lippe *et al.*, 1993). It was also reported that NO was directly or indirectly linked to immune complex-induced tissue injury (Mulligan *et al.*, 1991). The above findings have been confirmed using iNOS knockout mice (Wei *et al.*, 1995). In most cases, inhibition of NOS led to decreased inflammatory responses (Moncada *et al.*, 1991). Nitric oxide synthase can be inhibited by a group of guanidino-monosubstituted derivatives of L-arginine, including L-NMMA, L-NAME and L-N^G-amino arginine. These NO inhibitors have become a useful tool in the investigation of the physiological and pathological significance of NO (Moncada *et al.*, 1991; Moore *et al.*, 1991; Rees *et al.*, 1990a, 1990b)

There is evidence showing that the NO interacts with COX. It was reported that NO enhanced the generation of PGs and was suggested that the COX isoenzymes were potential receptor targets for NO in a renal inflammatory model (Salvemini *et al.*, 1994). It was also found that in acute pancreatitis inhibition of NOS caused a inhibition of 6-keto $PGF_{1\alpha}$ and TXB_2 generation, and PLT eicosanoid generation was mediated through a NO-dependent mechanism (Closa *et al.*, 1994).

Nitric oxide may play a role in inflammation in domestic animal species and the modulation of NO generation may have therapeutic value. However, there are little data in the literature about the role and regulation of NO in domestic animals. In this study we investigated the roles of NO

in acute inflammation, the effects of L-NAME on NO generation and the interaction of NO with eicosanoid generation using a tissue-cage inflammatory model in sheep *in vivo*.

5.2. Material and methods

5.2.1. Animals

Eight male sheep weighing 63 ± 3 (mean ± SD) kg and aged approximately 1.5 years old at the beginning of first cross-over were used. Hay and water were provided *ad libitum*.

5.2.2. Preparation of L-NAME injectable solution

The solution of L-NAME for i.v. administration was prepared as 20 % (W/V) in sterile distilled water. The solution was prepared within 24 h of injection in each cross-over occasion and stored at 0-4°C.

5.2.3. Experimental protocol

The experiments were carried out on a subcutaneous tissue-cage acute inflammatory model as described in Chapter 2. The cross-over experiment was coded as QM/ZC/03/95 and was started two months after the experiment coded QM/ZC/11/94 (Reported in Chapter 3) using the same tissue-cages and animals.

The study was carried out as a 5 way cross-over Latin square design, in which each sheep received an injection of carrageenan into a tissue cage 15 min before administration of test substances, sheep received i.v. 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) and CPF excipient as PLB, as a single rapid bolus, via the right jugular vein according to the cross-over design (see Table 4.1. the first 3 treatments were reported in Chapter 4.). A four-week interval was allowed between each occasion of the cross-over.

The sampling schedule and processing methods were described in Chapters 2 and 4. The values of TXB_2, PGE_2, LTB_4, haematology and the skin temperature over the cages were determined using the methods described in Chapter 2.

5.2.4. Quantification of NO

5.2.4.1. Reduction of nitrate to nitrite

The production of NO is associated with the generation of nitrite (NO_2^-) and nitrate, (NO_3^-). Nitrite may be detected by chemiluminescence, however in the exudate NO products include NO_3^- which must be reduced to NO_2^- for detection. In the present study, a nitrate reductase was used for the reduction.

All samples (exudate) or standards ($NaNO_2$ and $NaNO_3$ in serial concentrations of 12.5, 5.0, 2.5, and 1.25 μM) were prepared in duplicate, a PBS control was included. To each well of the plate, 30 μl of NO_2^- standard, or NO_3^- standard or sample was added. After this, 30 μl of reduction buffer [(containing 200μM of β-nicotinamide adenine dinucleotide phosphate (Sigma Ltd, Poole, Dorset, England), 20 μM of flavin adenine dinucleotide (Sigma Ltd, Poole, Dorset, England) and 0.5 units of nitrate reductase (Sigma Ltd, Poole, Dorset, England) in phosphate buffer saline] was added to each well. Plates were incubated at 37 °C for 30 min and then stored at 4 °C until injection. Where the injection is carried out some time after reduction, samples were stored at -20 °C.

5.2.4.2. Chemiluminescence analysis

The analysis was carried out using a chemiluminescence NO analyser (Model 2107, Dasibi Environmental Corporation, Glendale, California, USA) and a Chart recorder (Rikadenki, Yokogawa, Japan) was attached for the data record. In 100 mV switch, 1 scale in the paper was equal to 1 PPB and in 200 mV switch, 1 scale was equal to 2 PPB and so on.

The reaction solution was 100 ml (65 ml of acetic acid glacial and 35 ml of 6 % NaI). Prior to injection, the reaction solution was heated to boiling under a stream of nitrogen for 20 min until the trace in the record was stable.

The injection volume was 20 μl. The speed of injection was consistent and delivery was at the centre of the reaction solution surface.

5.2.4.3. Calculation of result

The scales were converted into PPB and standard concentrations were plotted against PPB. This generated a linear curve and the sample results were then calculated using linear regression. These procedures were carried out using a computer programmes compiled in Microsoft Excel 5.0.

Nitric oxide concentration = GROWTH(Standard NO concentrations, standard PPBs, sample PPB)

5.2.4.4. Statistics

The results were expressed as mean ± SE. The differences in the measurements collected at each time point between treatment groups, and between pre-treatment and post-treatment were analysed using ANOVA and Fisher's multiple comparisons as described in Chapter 2.

5.3. Results

5.3.1. Effects of L-NAME on NO production in inflammatory exudate

Nitrite was undetectable in exudate and transudate before nitrate was converted to nitrite. This indicated that the majority of the NO products in the tissue-cage fluids in sheep were present in the form of nitrate. The plot of nitrite (converted) concentrations versus times is given in Fig. 5.1. Before carrageenan injection, the concentration of nitrite in the tissue-cage fluids was detectable, with a mean value of 2.56 ± 0.32 μM (NO_2^-). Following intracaveal injection of

carrageenan, concentrations of exudate nitrite rose to a mean value of 4.93 ± 0.26 μM, increasing by 89.0 ± 8.8 % over a 96 h period and the concentrations were significantly elevated for up to 48 h ($P<0.01$) compared with the pre-values (20 min prior to carrageenan injection). Intravenous administration of L-NAME apparently increased the exudate NO concentration to a mean concentration of 5.18 ± 0.22 μM and the increase was significant at 2 h compared with the PLB-administered group ($P<0.05$).

5.3.2. Effects of L-NAME on increased inflammatory skin temperature

The effects of L-NAME on inflammatory heat production were estimated by measuring the changes in skin temperature over the carrageenan-induced and non-induced cages between the PLB and L-NAME treated groups. The results are given in Table 5.1. Following the injection of carrageenan into the tissue-cages, the skin temperature over the treated cages increased. In the PLB-treated group, the overall mean skin temperature over the carrageenan-treated cages was 0.43 ± 0.09 °C higher than that over the non-treated cages. A maximum increase of 1.33 ± 0.49 °C was achieved at 12 h, which was the only time that the increase achieved statistical significance compared with the pre-values ($P<0.05$). In the L-NAME administered group, the temperature changes were lower than in the PLB-treated group for up to 32 h and the overall mean temperature increase was 0.25 ± 0.09 °C. However no statistical significance ($P>0.05$) was achieved at any individual time point or between overall means of treatment groups.

5.3.3. Effects of L-NAME on serum TXB_2 generation

Mean ± SE serum TXB_2 concentrations are shown in Fig 5.2. and the percentage inhibition is given in Table 5.2. Analysis of variance showed that the difference in overall mean TXB_2 concentrations between the PLB and L-NAME treated groups were significant ($P<0.01$). The concentrations of serum TXB_2 in the L-NAME treated group were lower than those in the PLB treated group at all time points over a 72 h period although statistical significance was only achieved at 8 h ($P<0.01$). Compared with the pre-values, the L-NAME treated group showed a statistically significant decrease in serum TXB_2 generation for up to 48 h ($P<0.05$). However the inhibition of TXB_2 generation was only partial, indicated by a maximal percentage inhibition of 26.32 ± 6.81 occurring at 1 h and an overall mean percentage inhibition of 19.30 ± 1.92 %.

5.3.4. Effects of L-NAME on exudate PGE_2 generation

The injection of carrageenan induced the generation of exudate PGE_2 in the tissue-cages. In the PLB-administered group, PGE_2 concentration rose steadily from an undetectable level (below the limit of the analytical quantification) at time zero (15 min before carrageenan injection) to a C_{max} of 86.24 ± 11.75 ng/ml at 12 h following carrageenan injection. Compared with the controls, the increase in exudate PGE_2 was significant for up to 32 h ($P<0.05$). Intravenous administration of L-NAME increased exudate PGE_2 generation significantly ($P<0.05$). The overall mean exudate PGE_2 concentration over a 144 h period in the L-NAME treated group (45.91 ± 5.57 ng/ml) was significantly higher than that in PLB-treated group (35.35 ± 4.64 ng/ml) ($P<0.01$). At 12 h, a C_{max} for exudate PGE_2 of 112.60 ± 12.96 ng/ml was achieved, which was 23 % higher than in the PLB treated group. Compared with the PLB-treated group, the increase in exudate PGE_2 generation in the L-NAME treated group was significant at 12, 24, and 32 h. (Table 5.3.).

5.3.5. Effects of L-NAME on exudate LTB_4 generation

The plot of exudate LTB_4 concentration (mean ± SE) versus time following i.v. administration of PLB and L-NAME is presented in Fig. 5.3. In the PLB treated group, intracaveal carrageenan injection stimulated exudate LTB_4 generation from an undetectable level (below the limit of analytical quantification) to a C_{max} of 1.83 ± 0.44 ng/ml at 12 h. The concentrations of exudate LTB_4 were detectable over a 144 h period and the increase was significant from 8 to 120 h post carrageenan injection compared with the pre-value ($P<0.05$). Intravenous administration of L-NAME caused an increase in carrageenan-induced LTB_4 generation. The overall mean value (2-144 h) for the L-NAME treated group of 1.40 ± 0.13 ng/ml was significantly higher ($P<0.05$) than that for PLB-treated group (1.07 ± 0.073 ng/ml). The concentrations of exudate LTB_4 in the L-NAME treated group were higher than in the PLB treated group at most of the time points although the increase was not statistically significant at individual time points due to their large SE of the means ($P>0.05$).

5.3.6. Effects of L-NAME on white blood cell recruitment in exudate

The results for WBC numbers (mean ± SE, 10^9 cells/L) in the carrageenan-induced exudate following i.v. administration of PLB and L-NAME are given in Fig. 5.4.

Intracaveal injection of carrageenan caused a recruitment of WBCs in the inflammatory exudate in a time related fashion and a maximum WBC numbers of 17.63 ± 3.67 × 10^9 cells/L which occurred at 12 h. Of the WBC, 59.69 ± 4.63 % were neutrophils over a 48 h period. Intravenous administration of L-NAME enhanced the carrageenan-induced WBC accumulation and the overall mean WBC numbers of 14.88 ± 2.81 × 10^9 cells/L (in 4-48 h period) was significantly higher than that in the PLB treated group (10.17 ± 1.48 × 10^9 cells/L) ($P<0.01$). The exudate WBC numbers in the L-NAME treated group were higher than in the PLB treated group at most sampling time points but there was a significant increase at 24 h ($P<0.01$), at which time the maximum WBC numbers for the L-NAME treated group (37.47 ± 10.21 × 10^9 cells/L) was achieved.

5.3.7. Effects of L-NAME on WBC and PLT in venous blood

The mean ± SE of WBC and PLT numbers in venous blood are given in Table 5.4. Blood WBC numbers in the L-NAME treated group were higher than the values obtained before administration of L-NAME and in the PLB-treated group at all sampling points. Statistical significance was achieved at 4 and 24 h compared with the pre-administration values ($P<0.05$). The results also showed that L-NAME attenuated the PLT numbers in blood and the effect was significant at 4 h compared with the PLB-treated group ($P<0.05$).

Table 5.1. Changes in skin temperature (°C) over the cages following i.v. administration of PLB and L-NAME (25 mg/kg) in 8 sheep (mean ± SE)

Time (h)	PLB-treated group (°C, mean ± SE)	L-NAME-treated group (°C, mean ± SE)
Pre-values	0.08 ± 0.21	0.44 ± 0.27
1	0.29 ± 0.37	-0.19 ± 0.27
2	0.29 ± 0.27	0.06 ± 0.32
4	0.25 ± 0.43	0.14 ± 0.29
6	0.72 ± 0.18	0.29 ± 0.14
8	0.74 ± 0.18	0.38 ± 0.57
12	1.33 ± 0.49*	0.40 ± 0.42
24	0.43 ± 0.26	-0.07 ± 0.33
32	0.29 ± 0.26	0.06 ± 0.24
48	0.16 ± 0.26	0.29 ± 0.31
72	0.24 ± 0.30	0.04 ± 0.23
96	0.05 ± 0.41	0.54 ± 0.38
120	0.29 ± 0.29	0.72 ± 0.93
144	0.49 ± 0.31	0.58 ± 0.34

The values are the differences of the temperature between inflamed and non-inflamed tissue-cages (the temperature over the inflamed tissue-cage minus the temperature over the non-inflamed tissue-cages). Pre-values were the measurements 5 min prior to carrageenan injection. (*) $P<0.05$, compared with the pre-value.

Table 5.2. Changes in concentrations of serum TXB_2 following i.v. administration of PLB and L-NAME (25 mg/kg) in sheep (N=8, mean ± SE)

Time (h)	PLB-treated group (ng/ml, mean ± SE)	L-NAME-treated group (ng/ml, mean ± SE)
Pre-values	27.13 ± 1.86	29.97 ± 1.68
1	24.99 ± 2.48	22.45 ± 2.90*
2	26.36 ± 2.68	23.74 ± 2.42*
4	26.00 ± 2.25	23.81 ± 3.11*
6	25.72 ± 2.19	23.78 ± 2.13*
8	27.89 ± 2.29	22.96 ± 2.74*#
12	25.61 ± 2.16	23.97 ± 2.88*
24	26.71 ± 2.22	26.60 ± 2.16*
32	26.36 ± 2.59	26.16 ± 2.60*
48	24.92 ± 2.59	23.41 ± 2.24*
72	27.09 ± 1.66	26.78 ± 2.05

Pre-values were the measurements 5 min prior to carrageenan injection. (*) $P<0.05$, compared with the control value and (#) $P<0.05$, compared with the PLB treated group.

Table 5.3. Changes in concentrations of PGE_2 in exudate following i.v. administration of PLB and L-NAME (25 mg/kg) in 8 sheep (mean ± SE)

Times (h)	PLB-treated group (ng/ml, mean ± SE)	L-NAME-treated group (ng/ml, mean ± SE)
Pre-values	<0.8	<0.8
2	7.72 ± 6.81	4.95 ± 3.32
4	41.19 ± 13.51*	34.21 ± 11.63*
8	79.78 ± 11.18*	100.01 ± 13.16*
12	86.24 ± 11.75*	112.60 ± 12.96*#
24	62.45 ± 16.07*	83.07 ± 13.65*#
32	38.27 ± 9.78*	76.49 ± 13.60*#
48	7.99 ± 3.25	25.14 ± 11.90*
72	6.50 ± 3.59	9.23 ± 5.26
96	2.32 ± 1.02	5.72 ± 5.30
120	18.39 ± 18.39	8.92 ± 8.92
144	4.54 ± 4.04	9.09 ± 6.22

The measurements 5 min before carrageenan injection were used as pre-values. (*) $P<0.05$, compared with the control value and (#) $P<0.05$, compared with the PLB treated group.

Table 5.4. Changes in the numbers (10^9 cells /L) of WBC and PLT in venous blood following i.v. administration of PLB and L-NAME (25 mg/kg) in 8 sheep (mean ± SE)

Time	Blood WBC number		Blood PLT number	
(h)	PLB-treated	L-NAME-treated	PLB-treated	L-NAME-treated
Pre-values	7.68 ± 0.69	6.01 ± 1.07	179.13 ± 16.77	170.00 ± 28.35
4.00	7.57 ± 0.66	7.55 ± 0.88*	195.29 ± 18.65	171.40 ± 21.01#
24.00	7.68 ± 0.58	8.27 ± 0.42*	193.75 ± 22.69	189.63 ± 17.14
48.00	6.84 ± 0.59	7.11 ± 0.68	201.25 ± 18.87	196.63 ± 16.96

The measurements 5 min before carrageenan injection were used as pre-values. (*) $P<0.05$, compared with the controls and (#) $P<0.05$ compared with the PLB-treated group.

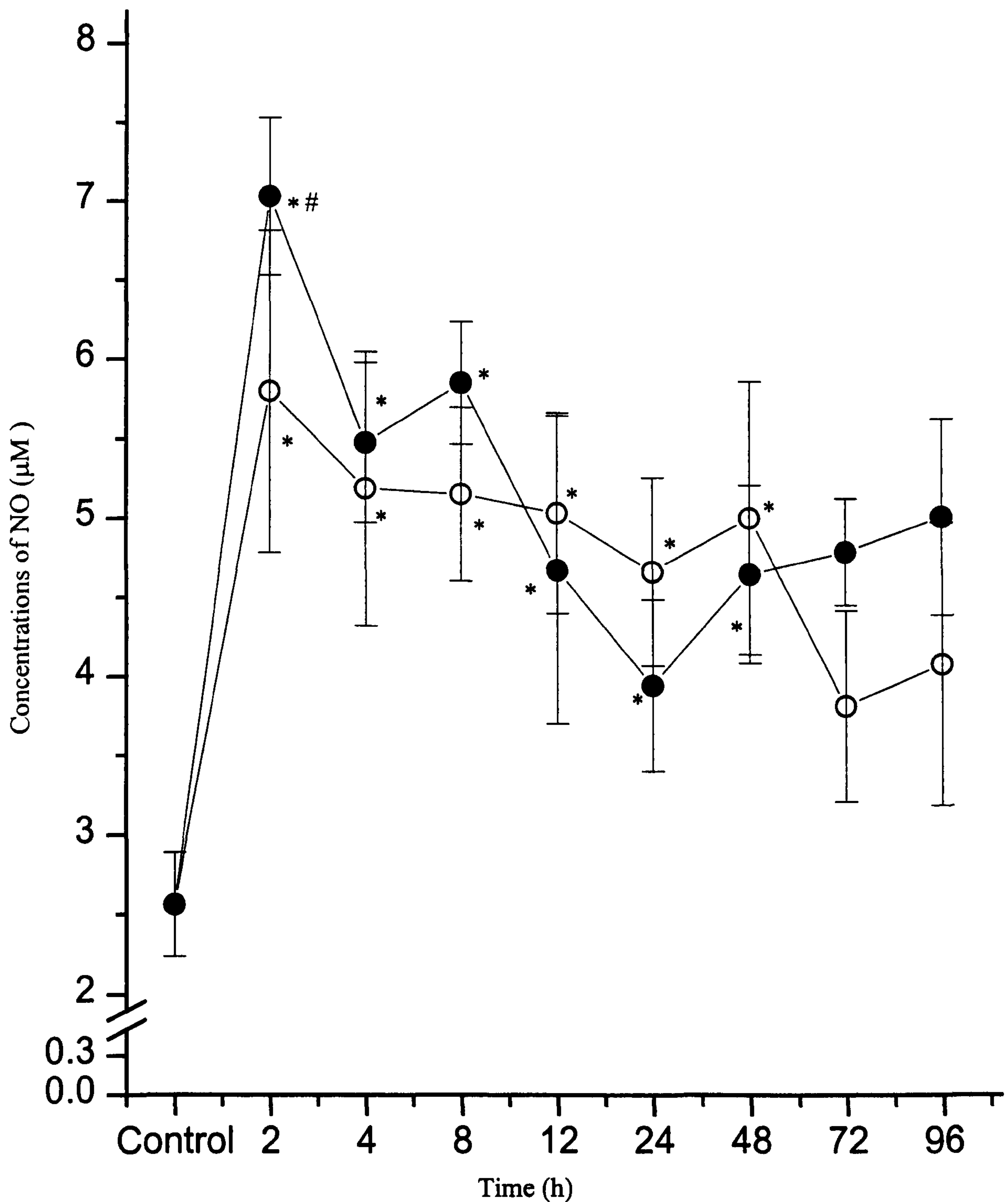


Fig. 5.1. Concentration versus time plot for exudate NO (μM) following i.v. administrations of L-NAME (–●–, 25 mg/kg) and PLB (–○–) in sheep (n=8, mean ± SE). The measurements prior to carrageenan injetction were used as controls. (*) P<0.05, compared with the control value and (#) P<0.05, compared with the PLB treated group.

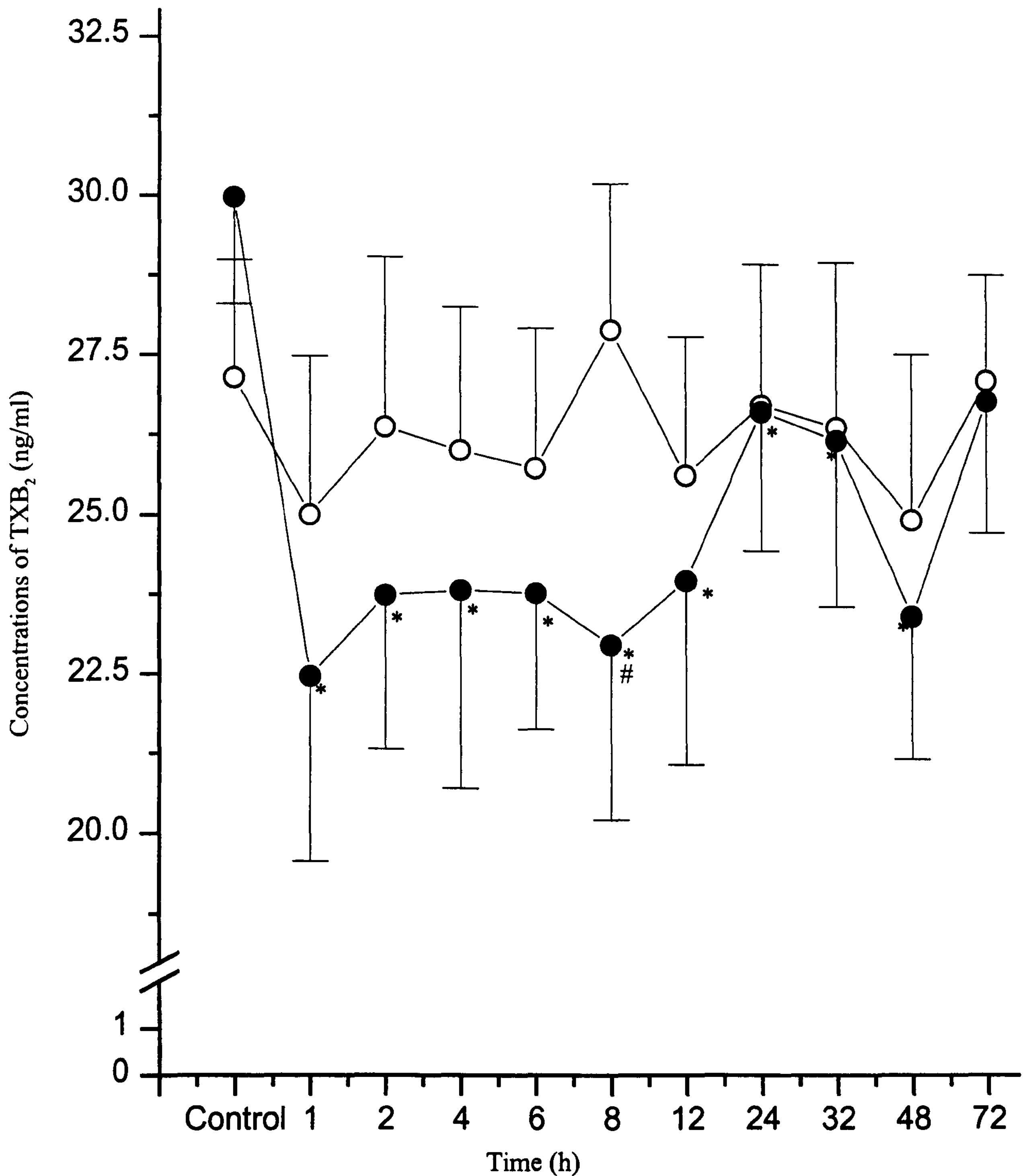


Fig. 5.2. Concentration versus time plot for serum TXB_2 generation following i.v. administrations of PLB (–○–) and L-NAME (–●–, 25 mg/kg) in sheep (n=8, mean ± SE). The measurements prior to carrageenan injection were used as controls. (*) P<0.05, compared with the control and (#) P<0.05 compared with the PLB-treated group.

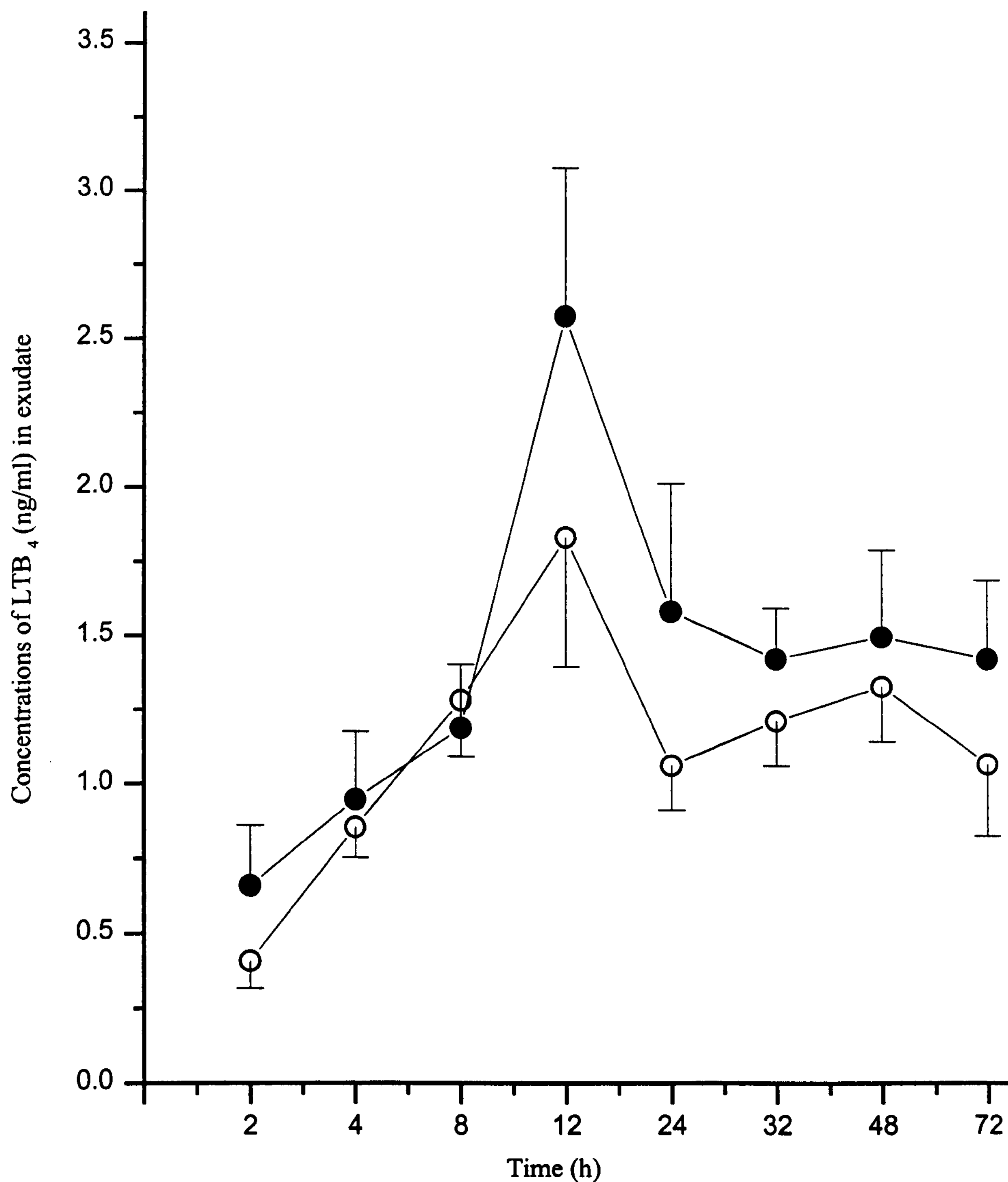


Fig. 5.3. Concentration versus time plot for exudate LTB_4 following i.v. administrations of L-NAME (–●–, 25 mg/kg) and PLB (–○–) in sheep (n=8, mean ± SE).

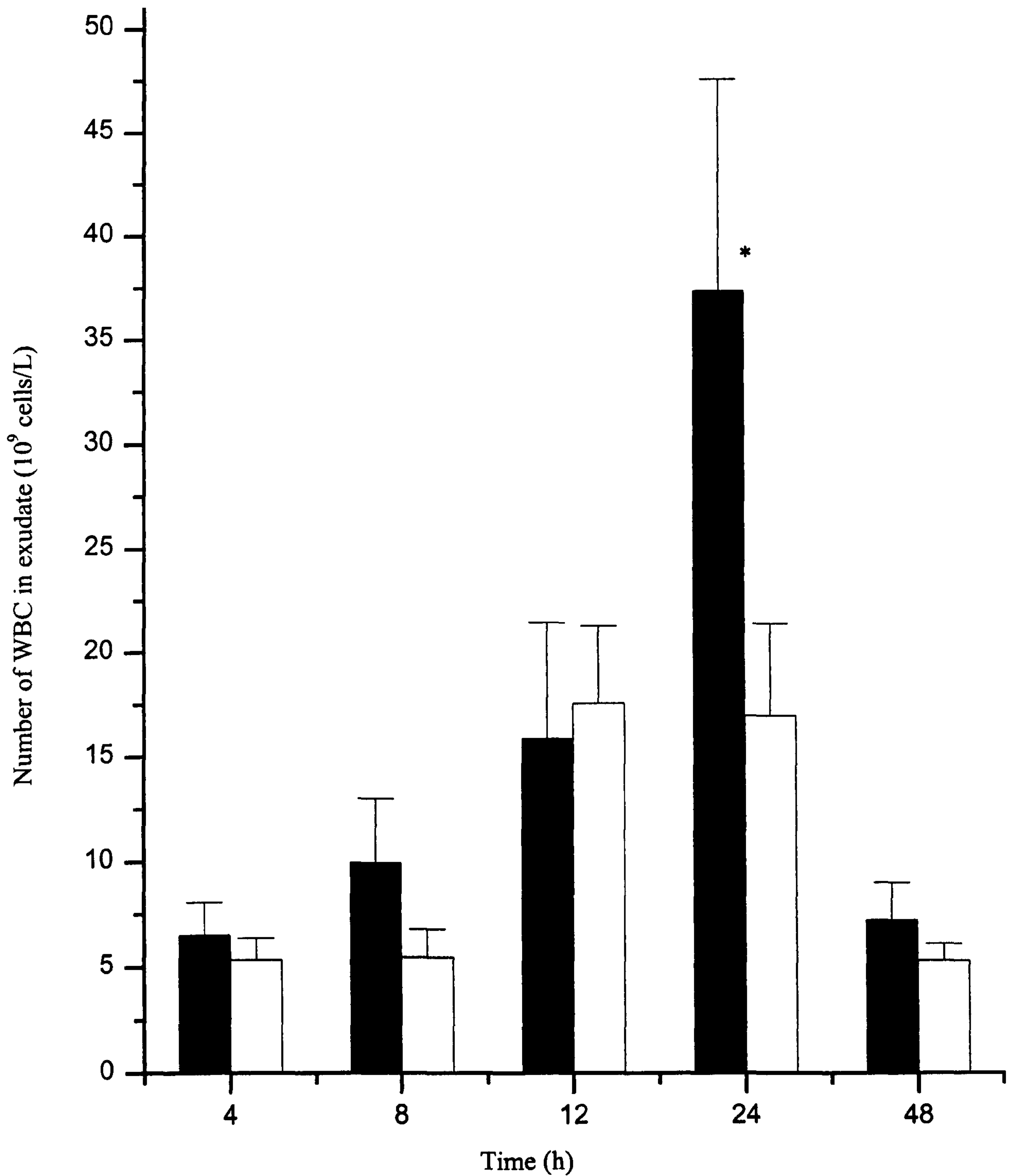


Fig. 5.4. Concentrations versus time plot for WBC counts in exudate following i.v. administrations of L-NAME (■, 25 mg/kg) and PLB (□) in sheep (n=8, mean ±SE). (*) $P<0.01$, compared with the PLB-treated group.

5.4. Discussion

Nitric oxide is derived from the guanidino nitrogen atom of L-arginine by the enzymatic action of NOS. There are at least two major isoforms of NOS. The constitutive enzyme (cNOS) is calcium-dependent and produces basal NO for the physiological process, such as homeostatic mechanisms. The inducible enzyme (iNOS) is calcium-independent and expressed following induction of various inflammatory agents (such as cytokines, LPS and carrageenan). Inducible NOS produces a large quantity of NO which is involved in defensive processes, especially inflammation (Moncada *et al.*, 1991). In the present study, prior to the injection of carrageenan into the cages the concentration of nitrate (including a small amount of nitrite) was 2.56 ± 0.32 μM and this may represent basal NO generated by cNOS. Following the injection of carrageenan, the concentration of nitrate (including nitrite) increased rapidly to a maximum value of 5.79 ± 0.01 μM at 2 h. This suggests that more than half of the NO in the inflammatory exudate was produced by iNOS and that the carrageenan tissue-cage model was appropriate for the study of NOS.

It has been reported that guanidino-monosubstituted derivatives of L-arginine, L-NAME, L-NMMA and L-N^G-amino arginine competitively inhibit both iNOS and cNOS *in vitro* and *in vivo* in rats and mice. Their effects include decrease in the formation of NO breakdown products (nitrate and nitrite) and inhibition of NO associated changes, such as inflammation, PLT aggregation and hypotensive responses (Rees *at al.*, 1990a, 1990b; Moncada *et al.*, 1991).

It was unexpected that in the present study in sheep i.v. administration of L-NAME caused an increase in NO formation in the exudate. This suggests surprising species differences. The mechanism underlying this finding is unknown. Previous reports illustrated that L-NAME, which is a potent inhibitor for endothelial NO synthesis (Rees *et al.*, 1990a), did not affect NO production in the neutrophil (McCall *et al.* 1990). In the present acute inflammatory model a large portion (59.69 ± 4.63 %) of the inflammatory cells accumulating in the exudate were neutrophils. But while this could explain a low response it can not explain the increase in NO generation following L-NAME administration. It is possible that L-NAME was rapidly

converted into L-arginine by metabolic enzyme systems in the sheep thus more substrate was available for the biosynthesis of NO. A metabolic study is required to confirm this hypothesis.

The initial intention in the present study was to use the inhibitory effect of L-NAME on NO generation, as a tool, to demonstrate the role of NO in acute inflammation. However the increase in NO production and the parallel changes in the inflammatory mediators does implicate NO as a mediator of inflammation in this species

Previous studies have indicated that inhibition of endogenous NO production by NOS inhibitors attenuates PG release and that an increase of NO generation by exogenously administering NO donors (glyceryl trinitrate and sodium nitroprusside) directly stimulates COX-2 activity to enhance PG generation. These results also suggested that COX isoenzymes are the receptor of NO (Salvemini *et al.*, 1993, 1994). The present study confirmed these findings since an increased NO production in the L-NAME treated group paralleled an enhanced PGE_2 generation in the exudate compared with the PLB-treated group. Prostaglandin E_2 and LTB_4 are formed from the same substrate, arachidonic acid, by the action of COX and 5-LOX, respectively. The phenomenon of metabolic shift between COX and LOX has been proposed (Higgs *et al.*, 1980; Kitchen *et al.*, 1985; Sedgwick *et al.*, 1987). Our previous studies (Chapters 3 and 4) illustrated that inhibition of COX activity by NSAIDs [FM and S(+)CPF] led to an increase in LTB_4 production in exudate by the metabolic diversion of arachidonic acid. However in the present study an increased NO production was accompanied by a rise in LTB_4 generation. This suggests that NO activated both COX and LOX or that NO may upregulate the activity of PLA_2, a proximal site in arachidonic acid metabolism since activation of PLA_2 releases arachidonic acid to provide an additional substrate for COX and LOX. This finding also suggests that both COX and LOX may be targets of NO. Prostaglandin E_2 and LTB_4 are important eicosanoids which initialise and maintain acute inflammation and the pro-inflammatory effects of NO may be amplified by increased PGE_2 and LTB_4 generation.

Nitric oxide has been demonstrated to have a role in regulation of PLT function. It inhibits PLT aggregation and adhesion to collagen fibrils, endothelial cell matrix and endothelial cell monolayers via a cyclic GMP-dependent mechanism (Radomski *et al.*, 1987a, 1987b, 1990; Moncada *et al.*, 1991). During the process of aggregation and adhesion, PLT produce and release

a number of bioactive substances, such as TXA_2, via various enzyme systems present in PLT. In the present study, i.v. administration of L-NAME caused an increased NO production in exudate and transudate, and an inhibition of TXB_2 (a breakdown product of TXA_2) in the serum. This also indicates an increased NO generation in serum and agrees with the findings in previous publications. It is possible that the effect of NO on COX is isoenzyme selective since PLT COX is COX-1 (Funk *et al.*, 1991) and carrageenan-induced exudate PGE_2 is predominantly synthesised by COX-2 (Masferrer *et al.*, 1994). It has been reported that COX isoenzymes are pharmacologically specific for some NSAIDs (Laneuville, *et al.*, 1994; Masferrer *et al.*, 1994; Williams & DuBois, 1996). However there is no published data on the isoenzyme selectivity of NO for COX-1 and COX-2.

Nitric oxide has been shown to be involved in mammalian immunological defence mechanisms (Moncada *et al.*, 1991; Mulligan *et al.*, 1991; Liew 1994; Wei *et al.*, 1995). It is a powerful vasodilator and NO generated by iNOS is cytotoxic for tumour cells and invasive organisms. The present study showed that NO production and leukocyte accumulation increased simultaneously in inflammatory exudate. The accumulation of leukocytes may result from increased vascular permeability and increased LTB_4 and PGE_2 concentration induced by NO. Vasodilatation facilitates infiltration of inflammatory cells, and LTB_4 is a powerful chemokinetic and chemotactic agent for those cells (Ford-Hutchinson *et al.* 1980). Furthermore vascular permeability can be caused by PGE_2. These findings agree with a previous report in which inhibition of NO synthase by L-NMMA inhibited neutrophil chemotaxis (Kaplan *et al.*, 1990).

In conclusion, NO appeared to play a role in acute inflammatory process in sheep by upregulating the activity of COX-2, 5-LOX and the accumulation of WBC. This suggests that combining iNOS-COX-2 inhibitors may prove to be a powerful strategy which may provide a broader antiinflammatory spectrum than NSAIDs. The finding that L-NAME increases NO production in inflammatory sites suggests a species difference in the metabolism of L-arginine analogues. Data concerning the inhibitory effects of L-arginine analogues on NO generation can not be extrapolated between animal species.

Chapter 6

Pharmacokinetics and pharmacodynamics of phenylbutazone and flunixin meglumine in donkeys

6.1. Introduction

Phenylbutazone and FM are the NSAIDs used extensively in equine medicine. They have been shown to have common PK and PD characteristics with other NSAIDs in experimental and domestic animal species (Vane 1971; Lees & Higgins 1984, 1986; Lees *et al.,* 1986, 1987a, 1987b, 1987c, 1991c; Tobin *et al*, 1986; McKellar *et al.*, 1989; Landoni *et al.*, 1995a). Previous results showed that PBZ and FM produce their therapeutic effects (antipyrexia, analgesia and antiinflammation) and side effects (including ulceration) by inhibition of the COX enzyme system and thus blockage of the synthesis of PGs and TXs which are the important inflammatory mediators for initialisation and maintenance of acute inflammation. Like other acidic NSAIDs, they accumulate in the acidic site of inflammation following administration so that when a dosage schedule is determined particular attention should be paid to their PK behaviour at the therapeutic site. Recently, the clinical uses of PBZ and FM have been extended to other animal species and other diseased conditions (Anderson., 1988; Selman., 1988; Kopcha & Ahl, 1989; Lees, 1989; Landoni *et al.*, 1995a).

However, the pharmacological use of PBZ and FM has not been described in donkeys (*Equus asinus*), and neither their action and nor disposition in this species have been studied. Previous studies have shown that NSAIDs exhibit marked species differences in PK parameters so that the data can not be transposed between species (McKellar *et al.,* 1990, 1994a, 1994b; Lees *et al.,* 1991d; Welsh *et al.*, 1993; Cunningham & Lees, 1994; Landoni *et al.*, 1995a, 1995b, 1996), and the potency of PBZ for COX inhibition is different between some animal species (P. Lees, personal communication. Also see Chapter 3 and 4).

The first purpose of the present study was to investigate the distribution and elimination of PBZ, OPBZ (an active metabolite of PBZ) and FM in plasma, inflamed tissue-cage fluid (exudate) and non-inflamed tissue-cage fluid (transudate). A second objective was to investigate the effects of PBZ and FM on COX isoenzymes, 12-LOX, inflammatory skin temperature and recruitment of

inflammatory cells. Finally the roles of COX and 12-LOX in carrageenan-induced acute inflammation in donkeys were investigated using the NSAIDs as a tool.

6.2. Materials and methods

6.2.1. Animals

Three female donkeys, named Cheeky, Frisky and Pinky, aged 7-15 years, weighing 160-200 kg at the beginning of the cross-over experiment were used. Hay and water were provided *ad libitum*.

6.2.2. Experimental protocol

The donkeys were prepared with a subcutaneously implanted tissue-cage on each side of the neck and acute inflammatory responses were initialised by intracaveal injection of 0.5 ml of 1% carrageenan 20 min prior to drug administration at -20 min and maintained by a further injection of carrageenan solution as described in Chapter 2.

The study was carried out as a three-way cross over such that each donkey was given i.v. PBZ at 4.4 mg/kg, FM at 1.1 mg/kg or PLB (physiological saline) at a volume equivalent to FM. A three week wash-out period was allowed between each cross-over occasion.

Plasma, blood, serum, transudate and exudate were harvested and processed as described in Chapter 2. The plasma samples were collected at 15 min before drug administration (-15 min), 5, 15, 30, 45 min and 1, 2, 3, 4, 6, 8, 12, 32, 24 and 48 h after drug administration. Serum samples for determination of TXB_2 concentrations were harvested at -15 min, 1, 2, 4, 6, 8, 12, 24, 32 and 48 h. Exudate and transudate were taken at -15 min (transudate only), 2, 4, 8, 12, 24, 32 and 48 h. Blood samples for haematological determination were collected at -15 min, 6, 24, 32 and 48 h, and exudate samples for haematological determination were harvested at 2, 4, 8, 12, 24, 32 and 48 h. Skin temperature over the cages was recorded at -20 min, 1, 2, 3, 4, 5, 6, 8, 12, 24, 32 and 48 h.

Phenylbutazone, OPBZ and FM concentrations were determined by HPLC using a chloroform extraction procedure as described in Chapter 2. Thromboxane B_2 and PGE_2 were measured using a dextran-coated charcoal RIA as described in Chapter 2 and 12-HETE was measured by RIA using a commercially available kit (AMI 12-HETE [3H] RIA Kit, Advanced Magnetics Cambridge, MA, USA). For the RIA, the samples were not extracted prior to the assay but diluted with RIA buffer as described previously. Haematological determination and skin temperature measurements were carried out as described in Chapter 2.

6.2.3. Pharmacokinetic and PD analysis

Pharmacokinetic and PD analysis was carried out using PCNONLIN 4.2 software as described in Chapter 2. Compartmental PK modelling was used for analysis of plasma data and the best fit model was determined using AIC (Yamaoka *et al* 1978). Non-compartmental modelling analyses were used for the transudate and exudate data. Areas under the zero- and first-moment curves were calculated from 0 to the time of last detectable sample. Pharmacodynamic analysis to determine the relationship between drug effects and drug concentration was fitted to a Sigmoid inhibitory model as described in Chapter 2.

6.2.4. Statistical analysis

Results are expressed as mean ± SE. A multiple factor ANOVA was carried out as described in Chapter 2 and Chapter 3 to determine differences of treatments, time points, sheep, sequences, periods, and their associated two factor interactions. The differences of the measurements collected at each time point between treatment groups and between pre-treatment and post-treatment were confirmed using Fisher's multiple comparisons following the ANOVA. Analysis of co-variance was carried out to establish the relationship between ambient temperature or pre-treatment values and the effects of treatments.

6.3. Results

6.3.1. Disposition of PBZ and OPBZ in plasma, exudate and transudate

The PK parameters (mean ± SE) for PBZ and OPBZ following i.v. administration of PBZ at 4.4 mg/kg are given in Table 6.1. and the concentration time curves are presented in Fig 6.1. and 6.2.

Following i.v. administration of PBZ, the C_p^0 of PBZ was 59.45 ± 2.04 μg/ml and this declined quickly. It was not detectable after 6 h in one animal and after 12 h in two animals. The concentration time data for PBZ in plasma was best fitted to a two compartment model with the equation: $C(t) = 40.98e^{-9.95t} + 18.46e^{-1.19t}$. Phenylbutazone was rapidly distributed and eliminated from plasma as indicated by a short $t_{\frac{1}{2}\alpha}$ of 0.09 ± 0.02 h, $t_{\frac{1}{2}\beta}$ of 0.63 ± 0.13 h and MRT of 0.69 ± 0.12 h. The mean V_{ss} of PBZ was small (147 ± 18 ml/kg). The conversion of PBZ to OPBZ was rapid, mean C_{max} of 4.03 ± 0.51 μg/ml for OPBZ in plasma occurred at 0.57 ± 0.10 h and the AUC ratio of OPBZ to PBZ was 67 %. The $t_{\frac{1}{2}\beta}$ of OPBZ was 1.81 ± 0.02 h which was about three times as long as that of PBZ.

The distribution of PBZ into exudate was greater than into transudate, indicated by the AUC ratio of 42 ± 3 % for exudate to plasma and of 26 ± 4 % for transudate to plasma. Phenylbutazone and OPBZ were eliminated much more slowly from exudate and transudate than from plasma and the values of MRT were considerably longer in cage fluids.

6.3.2. Disposition of FM in plasma, exudate and transudate

The mean ± SE PK parameters of FM are shown in Table 6.2. and the concentration time courses are illustrated in Fig 6.3.

Following i.v. administration, FM followed a bi-expenontial decay in plasma ($C = 17.20e^{-2.57t} + 5.19e^{-0.36t}$). Flunixin meglumine was detectable until 32 h in all of the animals. The distribution and elimination of FM were slower than those of PBZ, $t_{\frac{1}{2}\alpha}$ and $t_{\frac{1}{2}\beta}$ were 0.28 ± 0.02 h and 2.09

± 0.45 h, respectively. The V_{ss} and Cl_B were small, 171.07 ± 12.62 ml/kg and 50.27 ± 9.42 ml/kg/h, respectively.

Flunixin meglumine penetrated into tissue-cage fluids readily with a AUC ratio of 47 ± 13 % for exudate to plasma and of 55 ± 6 % for transudate to plasma. The C_{max} of FM in exudate was 1.30 ± 0.32 μg/ml which was slightly higher than in transudate (1.09 ± 0.26 μg/ml). The MRT of FM in exudate were about 5 times as long as that in plasma and slightly longer than that in transudate.

6.3.3. Effects of PBZ and FM on increased inflammatory skin temperature

Table 6.3. illustrates the temperature difference between the cage administered carrageenan and the cage without irritant following i.v. administration of PLB, PBZ and FM in donkeys.

Intracaveal injection of the mild irritant carrageenan increased mean skin temperature by 0.8 ± 0.2 °C compared with the cage without irritant during the 48 h period. A maximal increase of 1.6 ± 0.2 °C was recorded at 3 h. The temperature rose less in PBZ (0.4 ± 0.4 °C) and FM (0.4 ± 0.2 °C) treatment groups. Compared with the PLB-treated group, the overall temperature changes in FM-treated and PBZ-treated groups were significantly different ($P<0.05$) up to 8 h but not thereafter.

6.3.4. Effects of PBZ and FM on serum TXB_2 generation

The Effect of PBZ and FM on TXB_2 generation is illustrated in Fig. 6.4. and the values expressed in percentage inhibition are given in Table 6.4.

The overall means for serum TXB_2 concentrations in the PBZ treated group (35.97 ± 4.35 ng/ml) and the FM-treated group (17.08 ± 4.14 ng/ml) were significantly lower ($P<0.01$) than in the PLB-treated group (47.58 ± 3.97 ng/ml). The inhibition of serum TXB_2 generation by PBZ and FM was time-related ($P<0.01$) and this suggests a reversible inhibition of COX by PBZ and FM in this species.

Phenylbutazone produced a maximal reversible TXB_2 inhibition of 86.06 ± 2.66 % at 1 h and thereafter TXB_2 generation was gradually restored. Compared with the PLB-treated group and pre-values (15 min prior to drug administration), the inhibition was significant at 1, 2 and 6 h ($P<0.05$) and there was no significant rebound during a period of 48 h. Flunixin meglumine generated over 90 % inhibitory effect on serum TXB_2 for 6 h and maximal inhibition was 93.32 ± 1.56 % occurring at 6 h. Compared with the PLB-treated group and the pre-value, the inhibition was significant for up to 12 h ($P<0.05$), however there appeared to be a significant rebound effect after 32 h. The inhibitory effect of FM was significantly higher than that of PBZ from 4 to 12 h ($P<0.05$). In the plasma, the IC_{50} was 1.06 μg/ml and 1.86 μg/ml for PBZ and OPBZ, respectively, and the IC_{50} for FM was 0.11 μg/ml.

6.3.5. Effects of PBZ and FM on PGE_2 generation in exudate

Mean ± SE exudate PGE_2 concentrations following administration of PLB, PBZ and FM are shown in Fig. 6.5.

Following the injection of carrageenan into the tissue-cages, exudate PGE_2 concentrations increased steadily over a 48 h period. The maximal concentration was 3.33 ± 0.59 ng/ml achieved at 24 h and the increase was significant from 4 to 32 h ($P<0.05$). The overall mean PGE_2 concentrations in PBZ-administered group (1.48 ± 0.32 ng/ml) and FM-administered group (0.87 ± 0.35 ng/ml) were significantly lower ($P<0.05$) than in the PLB-administered group (2.07 ± 0.34 ng/ml). Compared with the PLB-administered group, PBZ and FM produced significant inhibition at 4 and 8 h ($P<0.05$). Flunixin meglumine produced higher inhibitory effect than PBZ during a 2-32 h period and statistical difference was determined at 8 h ($P<0.05$). In the exudate, the IC_{50} following PBZ treatment was 0.32 μg/ml PBZ and 0.33 μg/ml OPBZ and the IC_{50} for FM was 0.05 μg/ml.

6.3.6. Effects of PBZ and FM on 12-HETE in exudate

As shown in Table 6.5., neither PBZ nor FM modified the generation of 12-HETE in carrageenan-induced exudate.

6.3.7. Effects of PBZ and FM on the numbers of WBC and PLT

Neither PBZ nor FM modified the numbers of WBC and PLT in venous blood, or WBC in inflammatory exudate (Data not shown).

Table 6.1. Pharmacokinetic parameters of PBZ and OPBZ in plasma, exudate and transudate following i.v. administration of PBZ at 4.4 mg/kg in donkeys (n = 3, mean ± SE).

Parameters	Fluids	PBZ	OPBZ
C_p^0 (μg/ml)	Plasma	59.45 ± 2.04	NA
C_{max} (μg/ml)	Plasma	NA	4.03 ± 0.51
	Exudate	1.48 ± 0.56	0.72 ± 0.32
	Transudate	1.10 ± 0.38	0.85 ± 0.16
t_{max} (h) (Observed)	Plasma	NA	0.57 ± 0.098
	Exudate	5.33 ± 3.33	3.33 ± 0.67
	Transudate	2.00 ± 0.00	3.33 ± 0.67
$AUC_{0\text{-last}}$ (μg.h/ml)	Plasma	19.21 ± 2.11	12.86 ± 1.87
	Exudate	7.97 ± 0.77	7.02 ± 2.33
	Transudate	4.95 ± 0.54	6.43 ± 1.81
$AUC_{0\text{-}\infty}$ (μg.h/ml)	Plasma	20.53±1.27	NA
AUC_{ratio} (μg.h/ml)	Exudate:Plasma	0.42 ± 0.03	0.53 ± 0.11
	Transudate:Plasma	0.26 ± 0.04	0.48 ± 0.06
$t_{\frac{1}{2}}\alpha$ (h)	Plasma	0.09±0.02	NA
$t_{\frac{1}{2}}\beta$ (h)	Plasma	0.63 ± 0.13	1.81 ± 0.021
Cl_B (ml/h.kg)	Plasma	214.18 ± 18.04	NA
$MRT_{0\text{-last}}$ (h)	Plasma	0.69 ± 0.12	NA
	Exudate	10.17 ± 3.37	8.24 ± 0.96
	Transudate	10.87 ± 5.26	5.48 ± 0.46
$MRT_{0\text{-}\infty}$ (h)	Plasma	0.68±0.04	NA
V_{ss} (ml/kg)	Plasma	146.67 ± 17.63	NA

In plasma the PK analyses were carried out by compartmental modelling. The data of PBZ was fitted to a two compartment model with bolus iv injection and OPBZ was fitted to a one compartment model with first order input. In exudate and transudate the analysis was by non-compartmental modelling with extravascular administration. AUC ratios were calculated using $AUC_{0\text{-last}}$. NA = not applicable.

Table 6.2. Pharmacokinetic parameters of FM in plasma, exudate and transudate following i.v. administration of FM at 1.1 mg/kg in donkeys (n = 3, Mean ± SE).

Parameters	Plasma	Exudate	Transudate
C_p^0 (μg/ml)	22.39 ± 0.52	NA	NA
C_{max} (μg/ml)	NA	1.30 ± 0.32	1.09 ± 0.26
t_{max} (h) (Observed)	NA	2.63 ± 0.17	6.00 ± 3.06
AUC $_{0\text{-last}}$ (μg.h/ml)	23.63 ± 5.02	10.26 ± 2.17	13.38 ± 4.56
AUC $_{0\text{-}\infty}$ (μg.h/ml)	23.80 ± 4.99	NA	NA
AUCratio (Cage fluids/plasma)	NA	0.47 ± 0.13	0.55 ± 0.06
$t_{\frac{1}{2}\alpha}$ (h)	0.28 ± 0.02	NA	NA
$t_{\frac{1}{2}\beta}$ (h) (by non-compartment)	4.50 ± 0.09	NA	NA
$t_{\frac{1}{2}\beta}$ (h) (by compartment)	2.09 ± 0.45	NA	NA
$MRT_{0\text{-last}}$ (h)	3.42 ± 0.51	18.21 ± 3.30	14.33 ± 2.12
$MRT_{0\text{-}\infty}$ (h)	3.71 ± 0.42	NA	NA
Cl_B (ml/kg/h)	50.27 ± 9.42	NA	NA
V_{ss} (ml/kg)	171.07 ± 12.62	NA	NA

Concentration time data were fitted to a two compartment model and the data in tissue-cage fluids were analysed using non-compartmental modelling with extravascular administration. AUC ratios were calculated using $AUC_{0\text{-last}}$. NA = not applicable.

Table 6.3. The changes in skin temperature over the cages following i.v. administration of PLB, PBZ (4.4 mg/kg) and FM (1.1 mg/kg) in donkeys (n=3, mean ± SE).

Time (h)	PLB-treated group	PBZ-treated group	FM-treated group
Pre-treatment	0.3 ± 0.3	0.6 ± 1.0	-0.7 ± 0.3
1	0.5 ± 0.3	-0.2 ± 0.3	-0.5 ± 0.5
2	-0.1 ± 0.2	-0.1 ± 0.2	-0.3 ± 0.6
3	1.6 ± 0.2	0.3 ± 0.1	0.1 ± 0.8
4	1.5 ± 1.1	0.3 ± 0.4	-0.3 ± 0.5
6	0.4 ± 0.5	-0.2 ± 0.3	0.3 ± 0.4
8	0.4 ± 0.3	0.0 ± 0.4	0.8 ± 0.3
12	0.5 ± 0.3	0.5 ± 0.6	0.9 ± 0.3
24	1.5 ± 0.4	0.9 ± 0.5	0.6 ± 0.8
32	-0.3 ± 1.0	1.2 ± 0.8	0.5 ± 0.6
48	2.0 ± 1.3	1.7 ± 0.3	1.4 ± 1.3
Overall	0.8 ± 0.2	0.4 ± 0.4*	0.4 ± 0.2*

The values (°C) are the difference of skin temperature between carrageenan injected cages and the cages which did not receive carrageenan. * Significant difference compared with PLB-treated group ($P<0.05$).

Table 6.4. Percentage inhibition of serum TXB_2 following i.v. administration of PBZ (4.4 mg/kg) and FM (1.1 mg/kg) in donkeys (n = 3, Mean ± SE).

Time (h)	Percentage inhibition of TXB_2	
	PBZ-treated group	FM-treated group
1	86.06 ± 2.66*	91.47 ± 4.90*
2	69.48 ± 11.14*	91.08 ± 0.35*
4	24.05 ± 23.84	91.49 ± 1.04*
6	33.42 ± 11.87*	93.32 ± 1.56*
8	5.28 ± 12.14	85.45 ± 7.16*
12	7.66 ± 10.91	77.43 ± 5.27*
24	5.63 ± 7.77	15.97 ± 20.23
32	9.24 ± 7.67	-13.19 ± 17.47
48	-1.66 ± 13.20	-18.82 ± 15.67

* Significant difference compared with PLB-treated group ($P<0.05$)

Table 6.5. Concentrations of 12-HETE in exudate following i.v. administration of PLB, PBZ (4.4 mg/kg) and FM (1.1 mg/kg) in donkeys (n=3, mean ± SE).

Time (h)	Concentrations of 12-HETE acid (pg/ml)		
	PLB group	PBZ group	FM group
2	452 ± 64	418 ± 97	409 ± 99
4	653 ± 125	483 ± 126	562 ± 27
8	734 ± 168	508 ± 117	761 ± 98
12	400 ± 25	488 ± 85	460 ± 64
24	368 ± 69	336 ± 57	467 ± 102
32	482 ± 169	344 ± 28	497 ± 141
48	587 ± 202	346 ± 59	548 ± 190

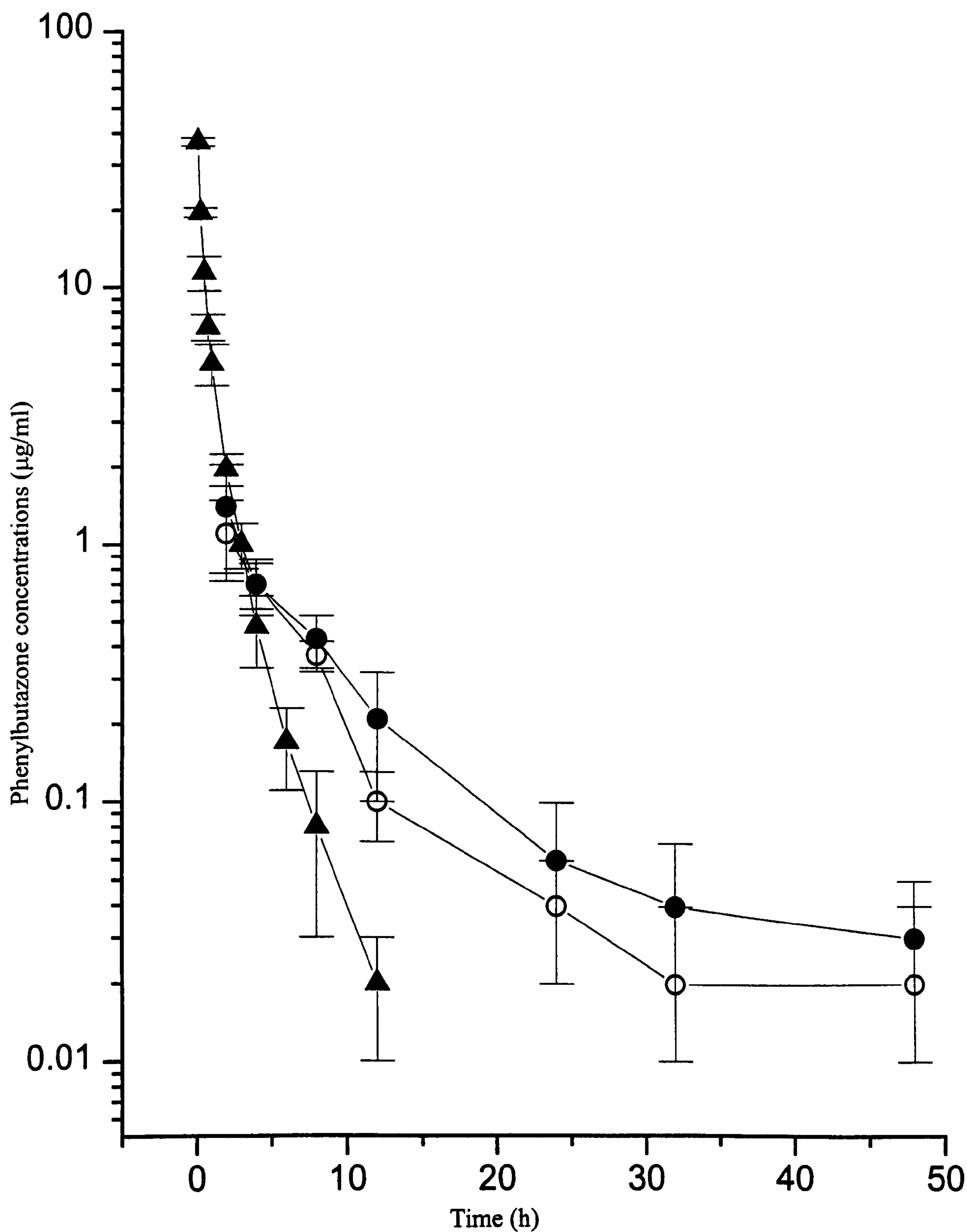


Fig. 6.1. Time course of PBZ in plasma (–▲–), exudate (–●–) and transudate (–○–) following i.v. adminstration of PBZ at 4.4 mg/kg in donkeys (n=3, mean ± SE).

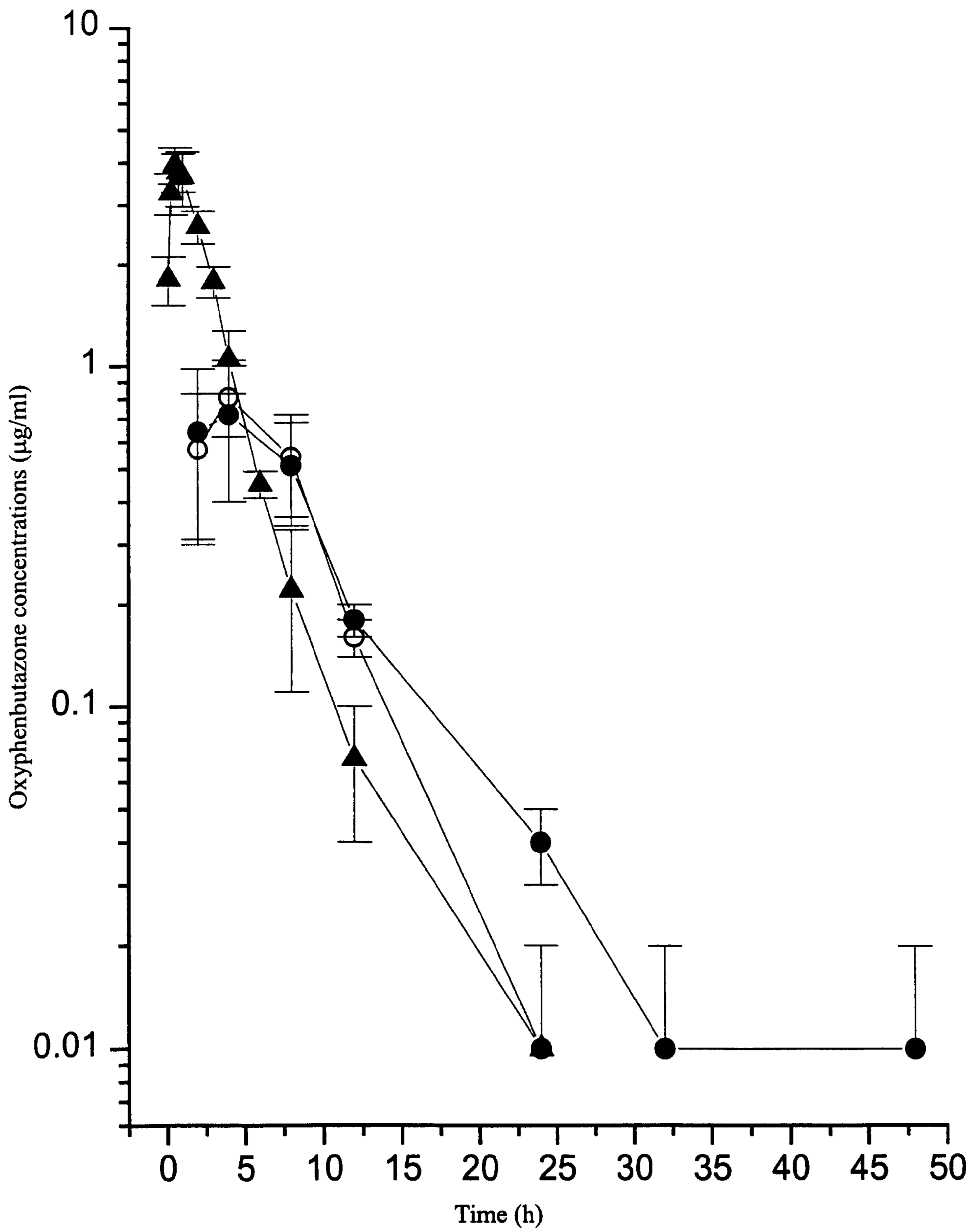


Fig. 6.2. Time courses of OPBZ in plasma (–▲–), exudate (–●–) and transudate (–○–) following i.v. administration of PBZ at 4.4 mg/kg in donkeys (n=3, mean ± SE).

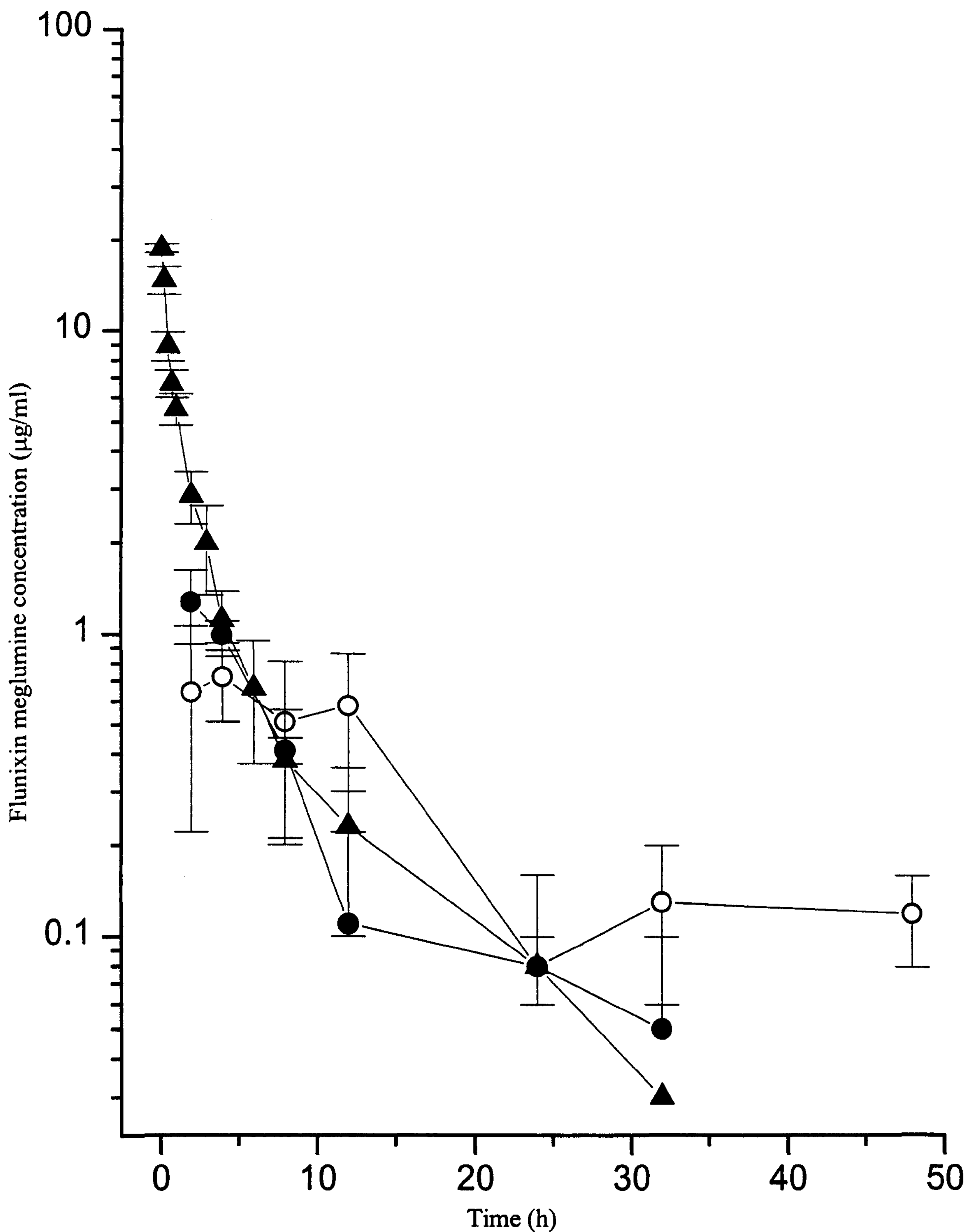


Fig. 6.3. Concentration versus time plot of FM in plasma (–▲–), exudate(–●–) and transudate (–○–) following i.v. administration of FM at 1.1 mg/kg in donkeys (n=3, mean ± SE).

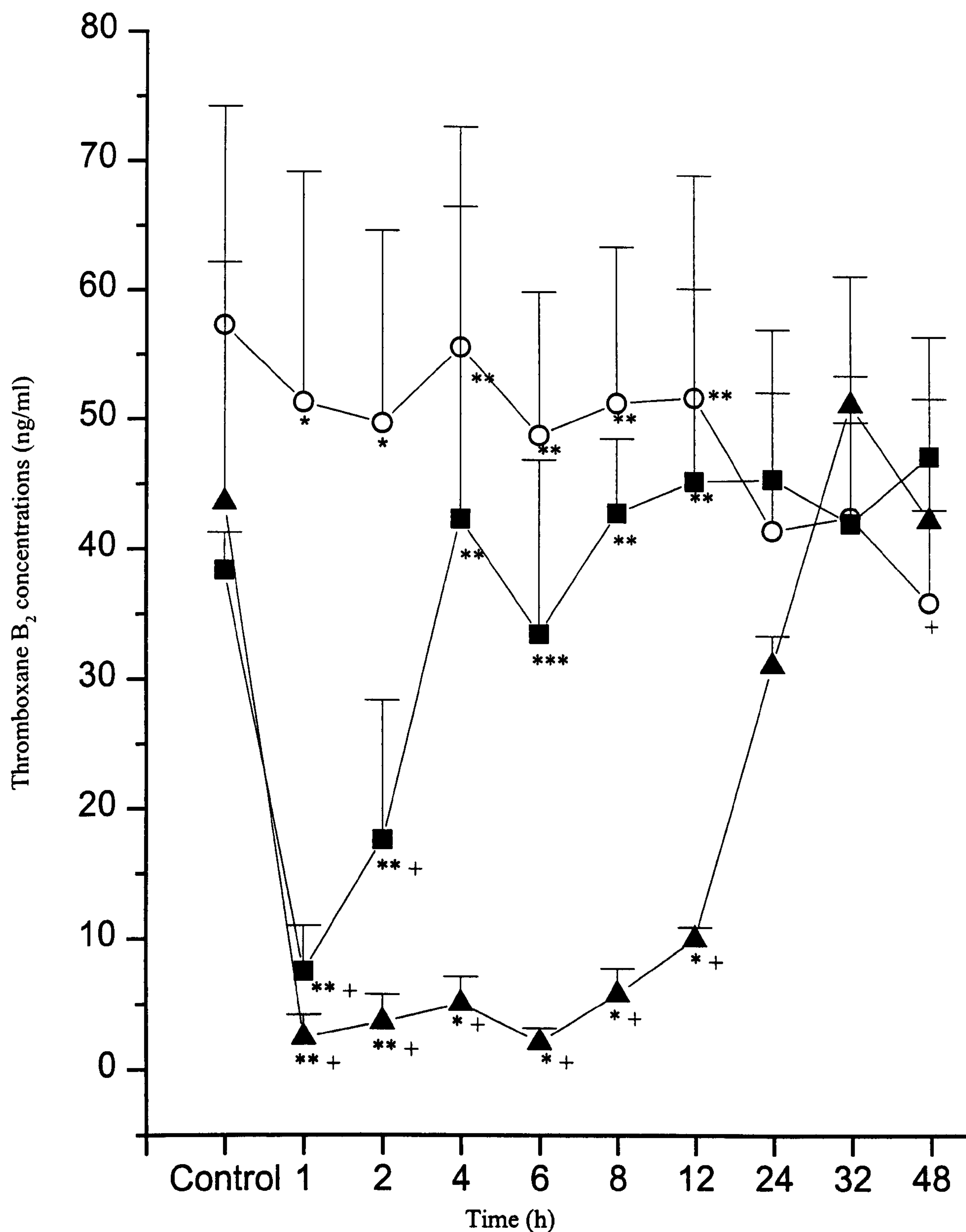


Fig. 6.4. Serum TXB_2 concentration versus time plot following i.v. adminstrations of PLB (saline, –○–), PBZ (4.4 mg/kg, –■–) and FM (1.1 mg/kg, –▲–) in donkeys (n=3, mean ± SE). The data points at each time with different numbers of asterisks are significantly different ($P<0.05$). (+) compared with the pre-value, $P<0.05$.

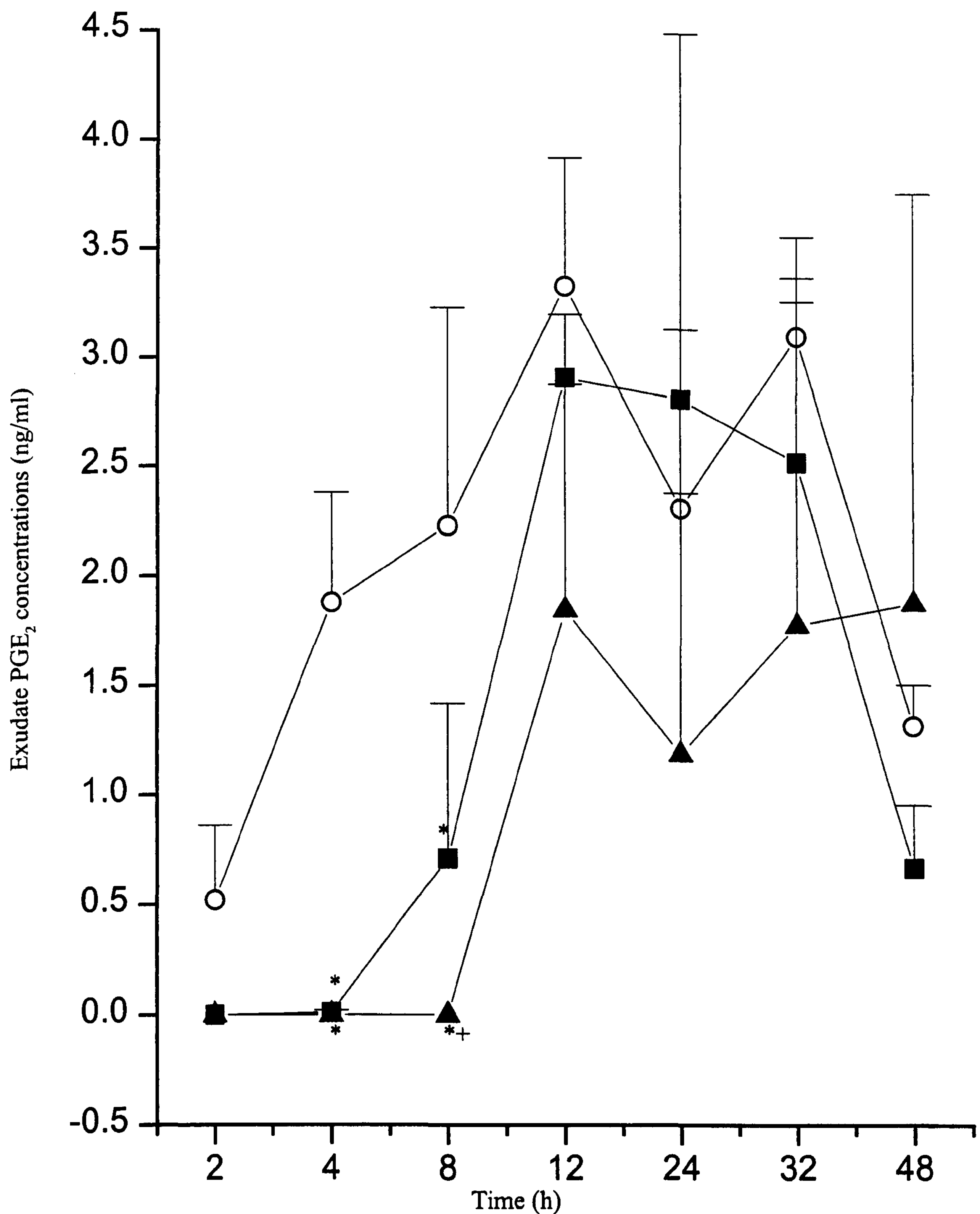


Fig. 6.5. Exudate PGE_2 concentration following i.v. adminstration of PLB (saline, –○–), PBZ (4.4 mg/kg, –■–) and FM (1.1 mg/kg, –▲–) in donkeys (n=3, mean ± SE). (*) compared with the PLB-treated group, $p<0.05$ and (+) compared with the PBZ-treated group, $P<0.05$.

6.4. Discussion

The PK of PBZ have been extensively studied in horses and it has been shown to produce a dose dependent kinetics (Tobin *et al.*, 1986). Following i.v. administration in horses at a single dose rate of 4.4 mg/kg, PBZ exhibited a $t_{\frac{1}{2}\beta}$ of 3.5-6.1 h (Piperno *et al.*, 1968; Lees *et al.*, 1986, 1987c; Maitho *et al.*, 1986). In the present study the elimination of PBZ was almost 10 times faster than that reported in the horse (ponies) following the same dose and route of administration (Lees *et al* 1986, Maitho *et al* 1986). The Cl_B of PBZ from Welsh mountain ponies was 25 - 26 ml/h.kg (Maitho *et al*, 1986) whereas in donkeys in the present study Cl_B was 214 ml/h.kg. Based on the relative areas under the curves for OPBZ and PBZ and the ratios of OPBZ to PBZ in donkeys (67 %) and ponies (8 %) (Lees *et al.*, 1986), it would appear that ring hydroxylation occurs more rapidly in the donkey which may account for the shorter half-life of PBZ, although it is also possible that OPBZ is eliminated more slowly in donkeys than horses.

The distribution of PBZ into tissue-cage fluids was slower and less extensive in donkeys compared to horses (Lees *et al.* , 1986, 1987a). In the present study, the C_{max} of PBZ was 1.48 ± 0.56 μg/ml in exudate and 1.10 ± 0.38 μg/ml in transudate while the same values in horse were 12.4 ± 1.3 μg/ml and 6.3 μg/ml in exudate and transudate, respectively. The ratios of AUC in donkeys was 42 ± 3 % for exudate to plasma and 26 ± 4 % for transudate to plasma, which were smaller than in horses (83 ± 4 % and 32 ± 4, respectively). This may be due to the quick elimination of PBZ and OPBZ in plasma of donkeys so that most of the drug molecules in plasma were cleared before they could readily penetrate into the tissue-cage fluids.

The plasma kinetic parameters for FM in donkeys in the present study were similar to those in horses following a same dose rate (1.1 mg/kg), administration routine and PK analysis method (compartmental modelling) (Lees *et al.*, 1987a). Thus $t_{\frac{1}{2}\beta}$ (2.09 ± 0.45 h in donkeys and 1.94 ± 0.24 h in horses), Cl_B (50.27 ± 9.42 ml/kg/h in donkeys and 57.3 ± 0.7 ml/kg/h in horses) and $AUC_{0\text{-last}}$ (23.63 ± 5.02 μg.h/ml in the donkeys and 19.43 ± 2.28 μg.h/ml in horses) were similar. It is interesting that in the present study $t_{\frac{1}{2}\beta}$ derived from non-compartmental modelling (4.50 ± 0.09 h) was twice as long as that derived from compartmental modelling using the same software (PCNONLIN 4.2). As mentioned in Chapter 3, $t_{\frac{1}{2}\beta}$ is a model-dependent parameter and when

comparisons are made, particular attention should be paid to the analysis methods since both compartmental and non-compartmental PK modelling have been widely accepted and used.

It was unexpected that FM did not show significant difference of AUC ratios between exudate to plasma and transudate to plasma in the present study. Penetration of FM into exudate in the present study in donkeys was similar to the data in horses reported previously (Lees & Higgins, 1984; Lees *et al.*, 1987c), indicated by an exudate C_{max} of 1.3 μg/ml in donkeys and of 0.9 μg/ml in horses. However in the horse study, the penetration of FM into transudate was lower than into exudate whereas in the present donkey study the penetration of FM into exudate and transudate was similar.

In the present study, PBZ and FM exerted significant inhibitory effects on serum TXB_2, exudate PGE_2 and skin temperature over the inflammatory cages but did not modify the generation of 12-HETE in exudate. This suggests that PBZ and FM produce their effects at least partially by reversible inhibition of COX. The percentage inhibition of exudate PGE_2 was still higher than 50 % at 8 h in the PBZ-treated group and 12 h in the FM-treated group, and were therefore much longer than the values of $t_{\frac{1}{2}\beta}$ in plasma. This indicates that PBZ and FM could exert therapeutic effects for longer than their plasma kinetics would suggest. This may be partially explained by their accumulation in the inflammatory sites. This was in agreement with previous reports in the horse (Lees & Higgins, 1984; Lees *et al.,* 1987c). The results also showed that the pathway for inhibition of PGE_2 generation was more sensitive than for TXB_2. In inflammatory exudate the IC_{50} (without correcting for binding to proteins) for PGE_2 was 0.32 μg/ml for PBZ (including 0.33 μg/ml of OPBZ) and 0.05 μg/ml for FM while the IC_{50} for TXB_2 generation was 1.06 μg/ml for PBZ (including 1.86 μg/ml of OPBZ) and 0.11 μg/ml for FM. Both PGE_2 and TXB_2 are derived from PGH_2 by the action of COX. However PGE_2 is formed via the endoperoxide isomerase and TXB_2 via TX synthetase pathways. It is possible that PBZ/OPBZ and FM block both COX and endoperoxide isomerase. It is more likely that PBZ, OPBZ and FM act differentially on COX-1 and COX-2 isoenzymes; blockade of *ex vivo* serum TXB_2 synthesis is likely to reflect drug action on the constitutive enzyme COX-1 in PLT whilst inhibition of the inducible enzyme COX-2 generated by inflammatory cells probably accounts for decreased generation of exudate PGE_2. In the study in sheep described in Chapter 3 the results for FM

agreed with the findings in the present study in donkeys while the results for PBZ were quite different. Previous studies in calves using the same inflammatory model showed that FM preferentially inhibited COX-1 (IC_{50} was 0.024 ± 0.004 μg/ml for serum TXB_2 and 0.074 ± 0.006 μg/ml for exudate PGE_2) (Landoni *et al.*, 1995a). Another study using human COX-1 and COX-2 cDNAs expressed in cultured cells illustrated that PBZ selectively inhibited COX-1 (Laneuville *et al.*, 1994). This may reflect species differences.

In conclusion, the present study demonstrated that PBZ and FM are effective inhibitors for COX and that they produce their pharmacological effects by inhibition of COX isoenzymes and thus the formation of PGs and TXs in donkeys. The PK of PBZ in donkeys were characterised by rapid elimination and were very different from those described in horses while FM in donkeys shares similar PK characteristics with horses when administered at the same dose rates. It seems likely that dosage interval of PBZ should be shorter in the donkeys whereas the dose of FM used in horses can be extended to donkeys. Tolerance studies are, however, required for both drugs in donkeys before firm recommendations can be made.

Chapter 7

Pharmacokinetic and Pharmacodynamic Studies on Phenylbutazone and Oxyphenbutazone in Goats

7.1. Introduction

Phenylbutazone is a NSAID which has not been extensively used in goats, however experimental studies and clinical investigations in cattle and other ruminants have indicated that NSAIDs are potentially useful drugs to treat arthritis, mastitis, pyrexia, endotoxaemia, viral respiratory diseases and other inflammatory conditions (Mercer & Teske, 1977; Giri *et al.,* 1984; Anderson *et al.,* 1985; Anderson 1988; Selman 1988; Kopcha & Ahl, 1989) and the disposition of PBZ has been determined in cattle (Eberhardson *et al.,* 1979; DeBacker *et al.,* 1980; Martin & Anderson, 1984; Lees *et al.,* 1988; Williams *et al.,* 1990). It is believed that eicosanoid generation and release are fundamental to the acute inflammatory process and that PBZ produces its effect by inhibiting the COX enzyme system which generates a number of eicosanoids, such as PGE_2 and TXA_2 (Vane, 1971; Lees *et al.,* 1987c). Previous studies on the fate of PBZ in goats illustrated that the elimination of PBZ from the plasma was slow and age-dependent following high dose i.v. administration of 10 mg/kg or 33 mg/kg (Boulos *et al.,* 1972; Eltom *et al.,* 1993). There are no published data on the bioavailability and mode action of PBZ in the goats.

In the present study, we have investigated the PK and TXB_2 inhibition in the goat following PBZ administered i.v. and p.o. at a dose rate of 4.4 mg/kg.

7.2. Materials and Methods

7.2.1 Animals and experimental protocol

Six mixed breed goats (3 males and 3 female) aged between 4-5 years and weighing between 45 and 92 kg were used for the experiment. All animals had *ad libitum* access to hay and water throughout the study. The study was carried out as a 3 way cross-over with two animals per treatment per period such that each animal received PBZ i.v. and p.o. and PLB treatment.

Animals were randomised for group by ballot and the interval between treatments was 3 weeks. Phenylbutazone for i.v. (Phenyzene, C-Vet Ltd, Braintree, Essex, England) or p.o (Equipalazone paste, Vet Drug Co., Hondon, England) administration was given at single dosage of 4.4 mg/kg. Placebo (0.9% NaCl) was administered i.v. at a volume of 1.0 ml.

Blood samples for measurement of PBZ and OPBZ concentration in the plasma were collected into 10 ml Lithium Heparin Monovettes (Sarstedt Ltd, Numbrecht, Germany) at 5 min before drug administration (-5 min) and 2, 5, 10, 15, 30, 45 min and 1, 2, 4, 6, 8, 12, 24, 32, 48, 72, 96, 120 and 144 h after drug administration. Blood was centrifuged at 1800 × g and 4 °C for 20 min and plasma was decanted into polystyrene tubes. The tubes were stored at -20 °C until analysis. Blood samples for TXB_2 assay were collected at -5 min and 1, 2, 4, 6, 8, 12, 24, 32, 48, 72, 96, 120 and 144 h and processed as described in Chapter 2.

7.2.2. Analyses of Samples.

Plasma concentrations of PBZ and OPBZ were estimated by HPLC and the measurement of TXB_2 was by RIA as described in Chapter 2.

7.2.3. Pharmacokinetic Analysis

Pharmacokinetic analysis was carried out as described in Chapter 2. Briefly, the plasma concentration-time data of PBZ and OPBZ for each individual animal were analysed by compartmental modelling using a non-linear estimation computer software programme, PCNONLIN 4.2 and the best fit model was determined as MAICE based on AIC (Yamaoka *et al.*, 1978). The AUC was calculated by the trapezoidal rule. The MRT and MAT were estimated using non-compartmental analyses. Body clearance and V_{ss} were estimated using compartmental approach. Bioavailability was corrected using $t_{\frac{1}{2}}\beta$, which corrected for the differences in $t_{\frac{1}{2}}\beta$ following each route of administration.

7.3. Results

7.3.1. Pharmacokinetics of PBZ and OPBZ in the plasma.

The PK parameters of PBZ and OPBZ following i.v. and p.o. administration are given in Table 7.1. The disposition of PBZ in plasma following i.v. administration at a dose rate of 4.4 mg/kg was best described by a two compartment model. The plasma concentration versus time plot is shown in Fig. 7.1. Phenylbutazone was eliminated slowly from plasma as indicated by a long $t_{\frac{1}{2}\beta}$ of 15.34 ± 1.15 h; a large AUC of 1229 μg.h/ml; a long MRT of 22 h and a slow Cl_B of 4.5 ml/kg/h. Following administration of PBZ paste, the disposition was best fitted by a one compartment model with first order input and output. The plasma concentration of PBZ versus time plot is given in Fig. 7.1. The absorption of PBZ was relatively slow with a MAT of 10.43 ± 3.51 h and a C_{max} of 27.23 ± 2.58 μg/ml occurring at 3.47 ± 0.39 h. In some animals, there were double peaks in the plasma concentration time course which may represent rumen by-pass by a proportion of the administered drug. The bioavailability was 82 ± 6% without correction and 61 ± 7 % corrected by $t_{\frac{1}{2}\beta}$. The elimination of PBZ was slower following p.o. than i.v. administration. The data for OPBZ disposition following i.v. and p.o. administration was best fitted to a one compartment model with first order input and output and the plasma concentration time curve of OPBZ is given in Fig. 7.1. This demonstrated a relatively low concentration of OPBZ in the plasma with C_{max} of 0.46 ± 0.09 μg/ml following p.o. administration. Oxyphenbutazone was not detectable in most of the goats 48 h after drug administration while PBZ was still detectable at 144 h after administration. The AUC ratios of OPBZ to PBZ were very small, 0.02 for i.v. and 0.016 for p.o. administration.

7.3.2. The effects of PBZ on serum TXB_2 generation

The mean ± SE concentration of TXB_2 in serum following i.v. and p.o. administration of PBZ is presented in Fig. 7.2. and the percentage inhibition is given in Table 7.2. The generation of TXB_2 by PLT COX was inhibited by PBZ in a time-related fashion. A maximal inhibition of 70.63 ±

11.96 % was achieved at 1 h following i.v. administration of PBZ and this decreased thereafter. Compared with TXB_2 concentration before drug administration, significant inhibition ($p<0.05$) occurred at 1, 2, 4, 6, 8 and 12 h. Following p.o. administration of PBZ, the generation of TXB_2 was also inhibited. The maximal inhibition, 64.34 ± 9.09 %, occurred at 2 h and inhibition was statistically significant ($p<0.05$) at 2, 4, 6, 8 and 12 h compared with the pre-values.

Table 7.1. Pharmacokinetic parameters of PBZ and OPBZ in plasma following i.v. and p.o. administration of PBZ at 4.4 mg/kg in goats (n=6, mean ± SE).

Parameters	Administration routes	Phenylbutazone	Oxyphenbutazone
C_{max} (μg/ml)	p.o.	27.23 ± 2.58	0.46 ± 0.09
C_p^0 (μg/ml)	i.v.	122.79 ± 14.33	NA
t_{max} (h)	p.o.	3.47 ± 0.39	8.24 ± 0.94
	i.v.	NA	1.47 ± 0.21
$AUC_{0-\infty}$ (μg.h/ml)	p.o.	981.43 ± 167.63	13.43 ± 2.36
	i.v.	1228.84 ± 219.41	19.37 ± 2.60
AUC_{ratio} (OPBZ/PBZ)	p.o.	0.016 ± 0.004	NA
	i.v.	0.02 ± 0.01	NA
F (Not corrected) %	p.o.	82 ± 6	NA
F (Corrected) %	p.o.	61 ± 7	NA
$t_{\frac{1}{2}k01}$ (h)	p.o.	0.69 ± 0.11	3.45 ± 0.84
$t_{\frac{1}{2}a}$ (h)	i.v.	0.29 ± 0.03	NA
$t_{\frac{1}{2}k10}$ (h)	p.o.	21.99 ± 3.32	14.31 ± 4.07
$t_{\frac{1}{2}\beta}$ (h)	i.v.	15.34 ± 1.15	21.66 ± 1.67
$MRT_{0-\infty}$ (h)	p.o.	32.00 ± 4.77	17.51 ± 6.81
	i.v..	21.58 ± 1.65	30.51 ± 2.59
$MAT_{0-\infty}$ (h)	p.o.	10.43 ± 3.51	NA
Cl_B (ml/kg/h)	i.v.	4.46 ± 1.12	NA
V_{ss} (ml/kg)	i.v.	87.99 ± 13.35	NA

NA = not applicable.

Table 7.2. The percentage inhibition of serum TXB_2 following i.v. and p.o. administration of PBZ at 4.4 mg/kg in goats (n=6, mean ± SE).

Time (h)	PBZ (i.v.)	PBZ (p.o.)
1	70.63 ± 11.96*	49.02 ± 15.41
2	61.82 ± 12.37*	64.34 ±9.09*
4	54.83 ± 11.80*	61.06 ± 11.83*
6	53.64 ± 12.02*	59.29 ± 7.17*
8	50.16 ± 12.35*	53.49 ± 10.28*
12	50.14 ± 12.08*	58.11 ± 8.25*
24	7.90 ± 8.28	35.19 ± 15.74
32	0.99 ± 12.10	44.17 ± 11.43
48	-9.74 ± 18.21	17.71 ± 16.88
72	-14.24 ± 16.46	27.25 ± 9.05
96	-13.27 ± 8.69	-12.20 ± 21.32
120	-39.60 ± 0.93	16.36 ± 48.15
144	-34.23 ± 22.10	29.83 ± 43.64

* $P<0.05$ Compared with pre-administration using one-way ANOVA with Dunnett's comparisons.

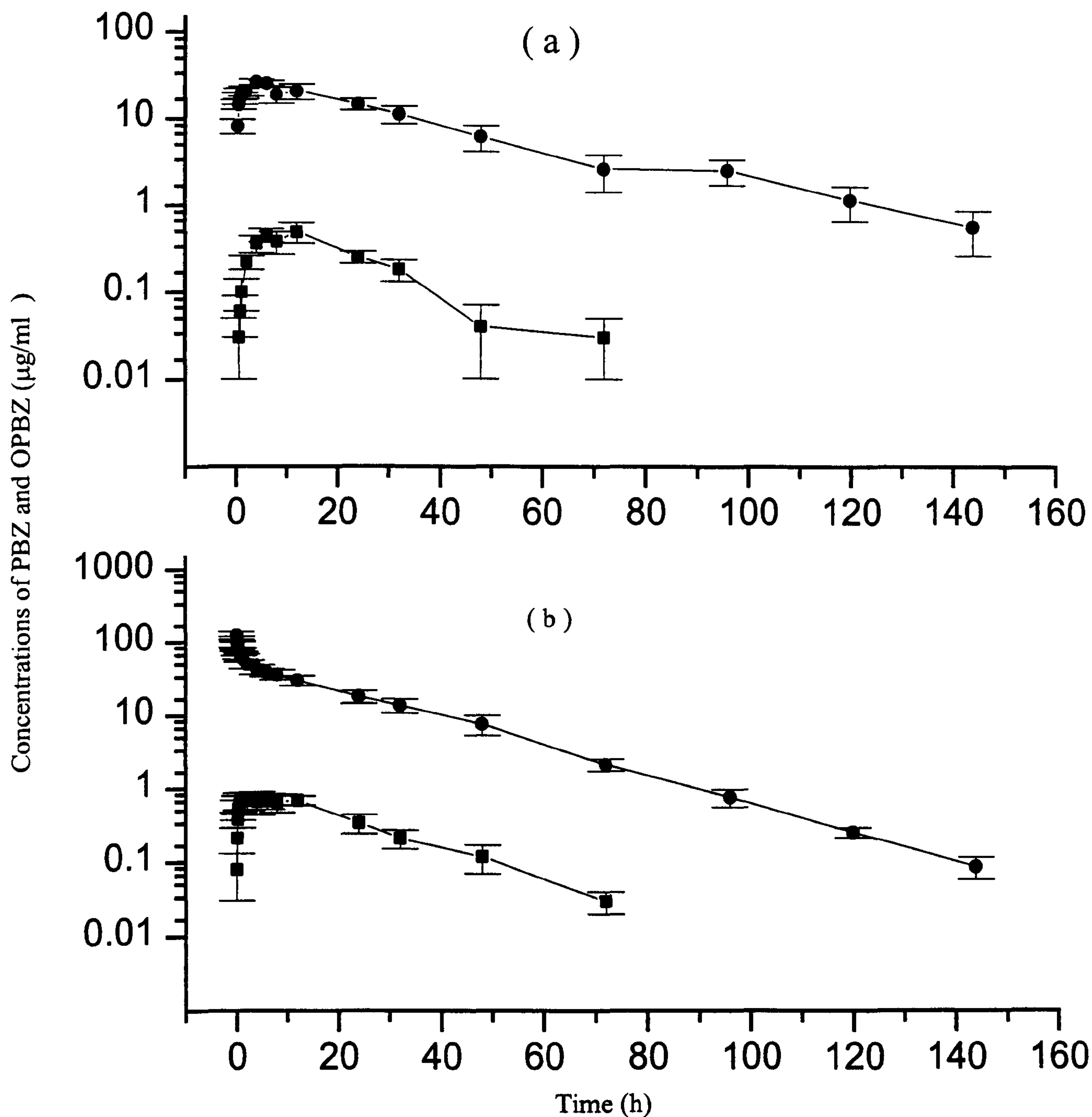


Fig 7.1. The concentration-time curves of PBZ (—●—) and OPBZ (—■—) following PBZ p.o. (a) and i.v. (b) administration at 4.4 mg/kg in goats (n=6, mean ± SE).

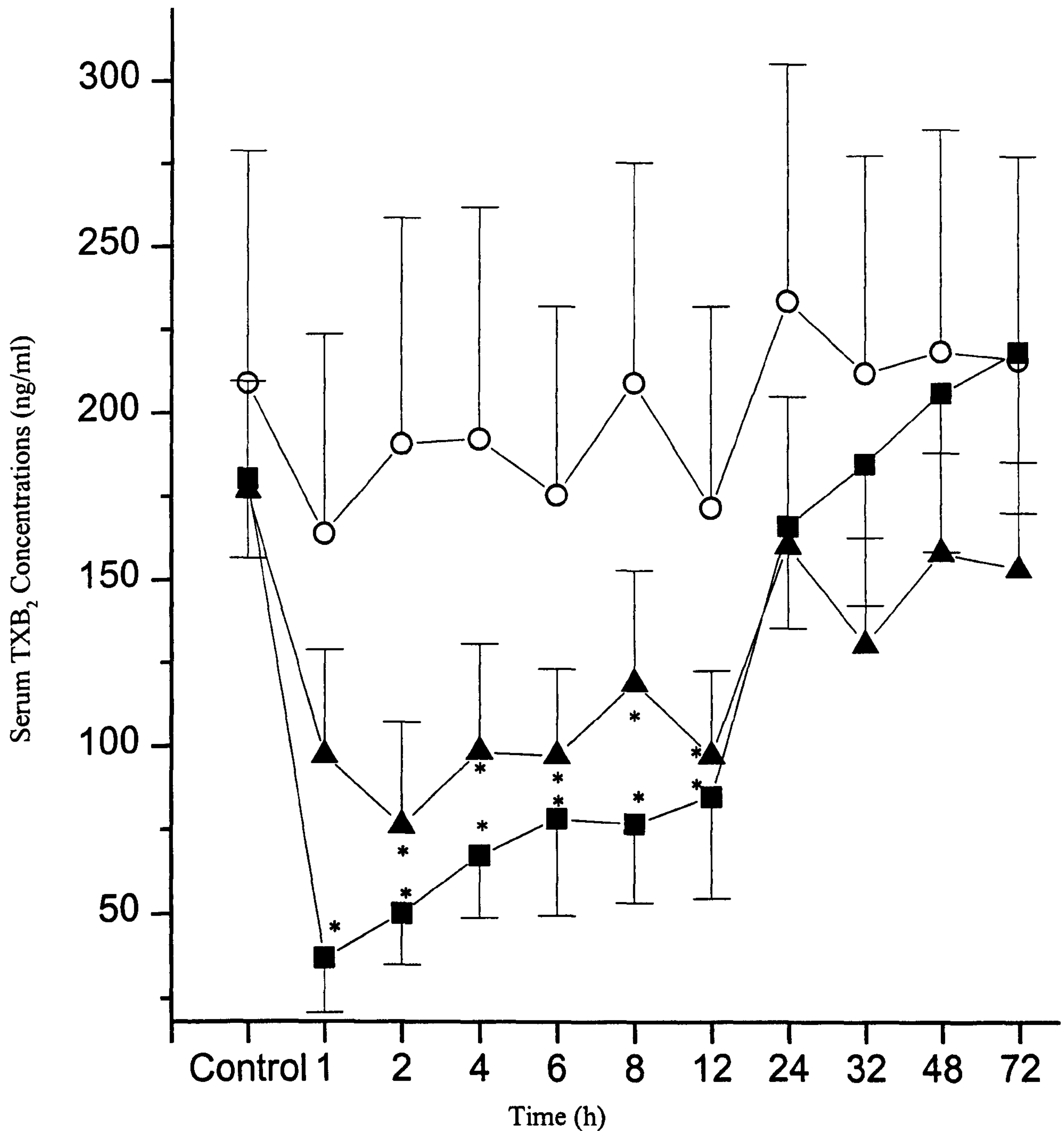


Fig 7.2. Changes in mean ± SE of serum TXB_2 concentrations (ng/ml) following i.v. (–■–, n=5) and oral (–▲–, n=6) administration of PBZ at 4.4 mg/kg in goats. (*) P< 0.05, compared with the pre-values. (–○–), PLB i.v.

7.4. Discussion

It has been reported that the elimination of PBZ from the plasma in ruminants was slower than in horses. A $t_{\frac{1}{2}\beta}$ of 15.9 ± 1.5 h was demonstrated in goats when a dose rate of 10 mg/kg was given i.v. (Eltom et al., 1993) and a $t_{\frac{1}{2}\beta}$ of 51.2 ± 6.8 h was obtained in cattle following i.v. administration of PBZ at 4.4 mg/kg (Lees *et al.*, 1988). In the horse, $t_{\frac{1}{2}\beta}$ was reported to be 6.11 ± 0.84 h following i.v. administration of 4.4 mg/kg (Lees *et al.*, 1987a, 1987c). Boulos *et al.*, (1972) gave PBZ to goats i.v. at dose rate of 33 mg/kg and obtained a $t_{\frac{1}{2}\beta}$ of 14.5 h. It has been shown that following PBZ at 10 mg/kg i.v. in the adult goat, the $AUC_{0-\infty}$ and $t_{\frac{1}{2}\beta}$ were 880 ± 84 μg.h/ml and 15.9 ± 1.5 h, respectively (Eltom *et al.*, 1993). In the present study, following 4.4 mg/kg i.v. in goats, the $AUC_{0-\infty}$ and $t_{\frac{1}{2}\beta}$ were 1228.8 ± 219.4 μg.h/ml and 15.3 ± 1.1 h, respectively. These findings suggest that the elimination of PBZ in goats is dose independent. It is not clear why the AUC following a 10 mg/kg dose rate was smaller than the AUC following the 4.4 mg/kg dose rate; but the studies were undertaken in different animals, in different laboratories and on different occasions.

Although PBZ was detectable at 144 h, the data for plasma PBZ concentrations over a sampling time of 2 min to 144 h did not permit a three compartment model to be fitted for i.v. administration or a two compartment model for p.o. administration. Moreover, V_{ss} was very low and this suggested that most of the drug entering the body stayed in the extra and transcellular compartment and that inter and intra cellular distribution was small. Concentrations of the hydroxylated metabolite, OPBZ, in the plasma were quite low and the AUC ratios of OPBZ to PBZ were very small, about 0.02:1 following i.v. or p.o. administration. In our HPLC traces, no peaks apart from PBZ and OPBZ were found in the plasma samples over a 20 min analytical time using a gradient mobile phase. It may be that hydroxylation in the liver was weak, or that excretion of hydroxylated metabolites was extremely rapid. Previous reports in cattle showed that hydroxylated metabolites of PBZ, OPBZ and γ-hydroxy PBZ in the urine but not PBZ itself were much higher than those in plasma for 168 h (Lees *et al.*, 1988).

The present study showed that absorption of PBZ following p.o. administration was relatively slow with a MAT of 10.43 ± 3.51 h and that the bioavailability (61%) was slightly higher than that reported in cattle (54%), (Lees *et al.*, 1988). The apparent $t_{\frac{1}{2}\beta}$ (p.o.) was about 7 h longer than the actual $t_{\frac{1}{2}\beta}$ (i.v.). This probably resulted from continued drug absorption processes in the gastrointestinal tract after C_{max} was achieved. The C_{max} following p.o. administration was about 5 times lower than that following i.v. administration and this probably explains a lower maximal TXB_2 inhibition after p.o. administration in the goat. The results suggested that because of the low bioavailability, higher dose rates may be required for p.o. administration to achieve and maintain adequate concentrations of active drug in plasma. Data for PBZ PK and PD in inflamed sites and tolerance data are required to confirm the appropriate dose for use in goats.

Eicosanoid generation is fundamental to the inflammatory process and the many components of inflammation, such as fever and pain, may be caused or exacerbated by eicosanoids. Inhibition of the formation of eicosanoids produces relief from the symptoms of inflammation. Arachidonic acid may be generated following several stimuli including thrombin during the blood clotting process. The arachidonic acid thus produced is further converted via the COX enzyme pathway into PGs and via TX synthetase to TXA_2 and its stable hydrolysate, TXB_2. A number of studies have illustrated that inhibition of TXB_2 generation in PLT by some NSAIDs correlates with inhibition of other COX products, such as PGE_2, in inflamed tissues (McKellar *et al.*, 1994b) since most of NSAID currently used in clinical practice are non-selective inhibitors for COX-1 and COX-2 isoenzymes (Laneuville *et al.*, 1994; Williams & DuBois, 1996). For instance, CPF which had little or no inhibitory effects on TXB_2 generation, failed to inhibit PGE_2 in inflamed exudate (McKellar *et al.*, 1994a). The generation of TX can be used as an indicator of PLT COX activity and may be used to estimate the likely inhibitory effect of NSAIDs on COX in inflamed tissues although this must be confirmed by the use of appropriate models of inflammation and in clinical trials. After the administration of PBZ to goats, the generation of TXB_2 in PLT was significantly inhibited ($p<0.05$) between 1 and 12 h (i.v.) and between 2 and 12 h (p.o.). It was partially or fully restored after 24 h following PBZ administration, which indicated the inhibition was reversible. The plasma concentration of PBZ was comparatively high at 24 h following administration, with a mean value of 17.99 μg/ml after i.v. and 14.54 μg/ml after p.o. administration, at which time TXB_2 concentrations were largely restored. Moreover, a very high plasma concentration of PBZ, 60.85 μg/ml which occurred at 1 h following PBZ i.v., failed to

abolish TXB_2 generation completely. Also in horse total PBZ plasma concentrations were much lower with the same dose rate of drug than in the goat, yet inhibition of TXB_2 was greater (Lees *et al.*, 1987a, 1987c). This may be due to species differences between goats and horses. Prediction of antiinflammatory effects of NSAIDs based on PLT COX inhibitory activity is however speculative. There are different isoforms of COX. The COX in PLT may be COX-1 which is a constitutive enzyme present in a wide range of tissues. Its function is believed to be related to physiological activities such as maintaining homeostasis. The COX in inflammatory sites may be COX-2, an inducible form of COX, which is responsible for generation of eicosanoids to develop and maintain acute inflammatory responses (Kujubu *et al.*, 1991; Simmons *et al.*, 1991; Xie *et al.*, 1991; Flecher *et al.*, 1992; Meade *et al.*, 1993). In studies in donkeys it was demonstrated that the total PBZ concentrations required to inhibit exudate PGE_2 in inflamed tissues were much lower than those required to inhibit serum TXB_2 generated by PLT COX (See Chapter 6). This suggested that an isoenzyme selectivity could be involved. Elimination of some NSAIDs from inflamed sites is much slower than from plasma and this may also contribute to the differences seen in plasma and inflammatory exudate (Lees *et al.*, 1986, 1987c; McKellar, *et al.*, 1994a, 1994b; Landoni *et al.*, 1995a, 1995b).

The present results suggest that PBZ may be a useful NSAID in goats when administrated at 4.4 mg/kg by the i.v. or p.o. routes although further studies are required to determine its safety in this species.

Chapter 8

Comparative pharmacokinetics of paracetamol (acetaminophen) and its sulphate and glucuronide metabolites in desert camels and goats.

8.1. Introduction

Paracetamol (acetaminophen) is a well recognised analgesic and antipyretic agent (Clark, 1979; Winter *et al.*, 1979) which has also been shown to have antiinflammatory activity in animals and man (Lokken & Skjelbred, 1980; Mburu *et al.*, 1988)

Its mode of action has not been fully characterised and although it is known to be a weak inhibitor of COX in the periphery (Mattammal *et al.*, 1979) it may have a more potent effect on enzymes in the central nervous system (Marshall *et al.*, 1987). The PK of PRT have been reported in several animal species including man (Rawlins *et al.*, 1977), dog (Omer & Mohammed, 1984) pig (Bailie *et al.*, 1987) and horse (Greenblatt & Engelking, 1988). In man and several other species PRT is metabolised primarily by glucuronide and sulphate conjugation (Prescott , 1980; Gregus *et al.*, 1988). However it has been shown that there are large interspecies differences in the metabolic fate of PRT. As well as the major detoxifying conjugation pathways to the glucuronide and sulphate metabolites there is a metabolic pathway which generates a toxic reactive electrophilic intermediate, N-acetyl-p-benzoquinoneimine (Fig. 8.1.). This metabolite is further biotransformed and detoxified to PRT glutathione conjugates including cysteine and cysteinylglycine conjugates (Gregus *et al.*, 1988). When the glutathione metabolic pathway is saturated, N-acetyl-p-benzoquinoneimine binds covalently to vital hepatic macromolecules which result in dose-dependent hepatic necrosis (Jollow *et al.*, 1973). The ratios of the detoxification and toxification pathways for the metabolism of PRT have been shown to correlate with species susceptibility to hepatotoxicity (Gregus *et al.*, 1988).

Previous reports have demonstrated differences between camels and goats in the activities of drug metabolising enzymes (El Sheikh *et al.*, 1986; El Sheikh *et al.*, 1988; El Sheikh *et al.*, 1991) which indicate that camels have lower metabolising capability for some drugs than goats. The

present study was carried out to determine the PK of PRT and its major detoxifying metabolites, glucuronide and sulphate, in desert camels and goats.

8.2. Materials and methods

8.2.1. Animals and experimental protocol

Six female desert camels weighing approximately 200 kg and aged two years and six female goats weighing approximately 25 kg and aged one year were used. Animals were given hay and water *ad libitum* and the diet was supplemented with pelleted concentrates and mineral blocks. Two experiments were run in parallel in two phases such that all the camels and goats were administered PRT i.m. and ten days later all the animals received PRT i.v. For each experiment PRT (pure compound) was dissolved in sterile normal saline (1 g/70 ml) and was administered at a dose rate of 5 mg/kg to camels or 10 mg/kg to goats. Blood (5 ml) was collected into heparinised tubes before and at 5 (i.v.) 10, 15 (i.v.) 20, 30, 40 (i.v.) 45 (i.m.), 60, 90, 120, 150, 210 (i.v.) and 240 (i.m.) min after administration of PRT. Blood was centrifuged at 900 × g for 10 min at 5 °C and plasma harvested and stored at -20 °C until analysed.

8.2.2. Measurement of drug concentrations

The concentrations of PRT, PRT glucuronide and PRT sulphate in plasma were determined by HPLC (Wang *et al.*, 1985). Paracetamol and PRT glucuronide were supplied by Sigma Ltd (Poole, Dorset, UK) and PRT sulphate was supplied by McNeil Consumer Products (Fort Washington, PA, USA). Plasma samples (0.25 ml) were precipitated with 6% perchloric acid and the supernatant removed for direct application to the HPLC system. A 25 cm × 4.6 mm C_{18} column (Alphasil 5 ODS) and guard column were used. The mobile phase was 7.5% acetonitrile in 50 mM phosphate buffer containing 50 mM Na_2SO_4 (pH 2.45) at a flow rate of 1.5 ml/min. The retention times for PRT glucuronide, PRT sulphate and PRT were 3.5, 5.3 and 6.5 min, respectively and recoveries and limits of quantification were 116.54 ± 2.49, 0.1 μg/ml (n=20), 98.48 ± 2.60, 0.1 μg/ml (n=20) and 98.97 ± 2.17, 0.1 μg/ml (n=20), respectively. Intra (n=18) and inter (n=4) assay co-efficients of variation were all less than 10% over the concentration ranges 0.1 - 2.5 μg/ml (PRT), 0.2 - 75 μg/ml (PRT glucuronide), 0.2 - 25 μg/ml (PRT sulphate).

8.2.3. Data Analysis

The data from each individual animal for the parent PRT and the metabolites studied were analysed using non-compartmental model analysis (PCNONLIN version 4.2) as described in Chapter 2. This programme used algorithms to determine the first-order rate constant associated with the terminal (log-linear) portion of the curve (Beta) for the estimation of area under the concentration time curve from zero time to infinity (Dunne, 1985). The parameters derived from non-compartmental modelling were determined using a linear trapezoidal method as described in Chapter 2. Bioavailability was estimated from the equation described by Gibaldi and Perrier (1982b):

$$F = \frac{AUC_{oral} \times t_{\frac{1}{2}\beta_{i.v.}}}{AUC_{i.v}.x t_{\frac{1}{2}\beta oral}}$$

This method assumes that a change in $t_{\frac{1}{2}}\beta$ from one study period to the next in the same subject reflects a change in clearance and is not mediated by a change in apparent volume of distribution.

Statistical comparisons of PK parameters within each animal species were made using Wilcoxon signed rank test and between each species using Mann Whitney U-test. Differences were considered significant when $P < 0.05$.

8.3. Results

The plasma concentration versus time curves for PRT and its glucuronide and sulphate metabolites following administration of PRT by the i.v. and i.m. routes to camels and goats are shown in Figs. 8.2 - 8.5. Parent PRT concentrations fell rapidly in both animal species and in the camel the sulphate metabolite initially predominated in plasma whereas in the goat the glucuronide metabolite was dominant in plasma. In the camel the glucuronide metabolite formed a large component of the plasma metabolite profile and from approximately 120 min following i.v. administration the glucuronide predominated. Following i.m. administration sulphate and glucuronide plasma concentration curves predominated and converged such that at the last

sampling time (210 min) mean sulphate and glucuronide concentrations were similar. In the goat the sulphate metabolite formed a minor component of the total plasma metabolite disposition profile (approximately 3.89 % of the $AUC_{0\text{-}last}$ of the glucuronide).

The PK parameters for PRT following i.v. administration in camels and goats are given in Table 8.1. Paracetamol was cleared much more slowly (21.9 ± 1.4 ml/min.kg) in camels than in goats (52.8 ± 7.3 ml/min.kg) although the V_{ss} were similar (1210 ± 42 ml/kg camels vs 1418 ± 138 ml/kg goats) in both species.

The PK data for the glucuronide and sulphate metabolites following i.v. administration of PRT to camels and goats are given in Table 8.2. Since a large proportion of the metabolite disposition curve appeared to lie after the last sampling time an accurate description of β (the slope of the elimination curve) could not be determined, consequently all values were determined from zero time to the last sampling time. In camels the time to C_{max} was twice as long for the glucuronide metabolite than the sulphate, however the MRT of the glucuronide was also longer and it is apparent that the AUC for the glucuronide would be relatively greater compared to the sulphate if the disposition curves had been taken to infinity. In three animals for which β could be determined $AUC_{0\text{-}\infty}$ was 3241.6 ± 726.4 μg.min/ml for the glucuronide and 1514.8 ± 266.1 μg.min/ml for the sulphate.

In goats the plasma disposition curves were more completely defined and consequently the PK parameters from zero time to last sample are more accurate. In the goat the glucuronide metabolite predominated in plasma and produced an $AUC_{0\text{-}last}$ which was 3.8 times as large as that achieved in the camel (1.9 times when corrected for dose of PRT administered). The sulphate metabolite formed a very much smaller component of the total plasma metabolite profile in the goat than in the camel.

The PK data for PRT and both measured metabolites following i.m. administration to camels and goats are given in Table 8.3. The plasma metabolite profiles were qualitatively similar following i.m. administration compared to i.v. administration with an absorption phase for parent drug and a longer lag phase for the metabolites attributable to absorption of parent drug. The MAT was longer in camels than goats. The bioavailability could only be determined accurately for five

camels and four goats in which i.v. and i.m. Beta values could be estimated. In these animals $F_{0\text{-}last}$ using the $t_{\frac{1}{2}}\beta$ correction method for PRT given by the i.m. route was 71 ± 17% in goats and 105 ± 26% in camels.

8.4. Discussion

In the present study large differences in the plasma PK of PRT and its metabolites in camels and goats were observed. In the camel sulphate metabolism predominated and although the concentrations of glucuronide were higher than sulphate from 120 h after i.v. administration this appeared to be due to slower excretion of the glucuronide. In goats the glucuronide metabolite predominated in plasma. The plasma metabolite concentrations depend upon their rate of generation, volume of distribution and clearance. Thus a metabolite could be dominant (represent the main mass fraction in terms of PRT transformation) and have a lower plasma concentration than a metabolite formed to a lesser extent but with a smaller volume of distribution and/or lower clearance. It is therefore important to take this into account when considering the relative metabolite concentrations in plasma.

In both species the parent PRT had a very rapid clearance (21.9 ± 1.4 ml/min.kg - camel vs. 52.8 ± 7.3 ml/min.kg - goat) and the differences in clearance between species were significant ($P < 0.01$). The extent of distribution was similar in both species. The clearance of PRT was faster in both ruminant species than in man (5.45 ± 0.20 ml/min.kg) and horses (4.61 ± 1.29 ml/min.kg) (Prescott,1980; Greenblatt and Engelking, 1988) and since very low concentrations of PRT (<2.5 μg/ml) were present from 50 min after administration by either route in both species, it may be that the analgesic and antipyretic effects of the drug are short in these species.

Table 8.1. Pharmacokinetic data (mean ± SE) for PRT following i.v. administration to camels (n = 6) and goats (n = 5).

Parameters	Camel	Goats
Dose (mg/kg)	5	10
β (min^{-1})	0.016 ± 0.001	0.123 ± 0.092
$AUC_{0\text{-last}}$ (μg.min/ml)	220.6 ± 11.8	202.4 ± 28.3
$AUC_{0\text{-}\infty}$ (μg.min/ml)	232.3 ± 13.1	205.3 ± 29.3
$MRT_{0\text{-last}}$ (min)	46.3 ± 2.0	26.2 ± 2.3
$MRT_{0\text{-}\infty}$ (min)	55.9 ± 2.7	27.8 ± 2.6
Cl_B (ml/min.kg)	21.9 ± 1.4	52.8 ± 7.3
V_{ss} (ml/kg)	1210 ± 42	1418 ± 138

Cl_B = Dose/AUC; V_{ss}= $Cl_B \times$ MRT

Table 8.2. Pharmacokinetic data (mean ± SE) for PRT major metabolites following i.v. administration of PRT to camels at 5 mg/kg (n = 6) and goats at 10 mg/kg (n = 6).

Parameter	Camels		Goats	
	B Glucuronide	Sulphate	B Glucuronide	Sulphate
t_{max} (min)	34.17 ± 6.64	15.83 ± 2.01	18.33 ± 1.67	13.33 ± 4.01
C_{max} (μg/ml)	9.23 ± 1.34	17.87 ± 1.34	55.05 ± 7.0	2.55 ± 0.44
$AUC_{0\text{-last}}$ (μg.min/ml)	1145.8 ± 124.7	1452.8 ± 357.9	4391.1 ± 551.4	170.9 ± 35.8
MRT (min)	82.7 ± 0.6	66.3 ± 5.5	60.7 ± 1.5	5.29 ± 5.7

Table 8.3. Pharmacokinetic data (mean ± SE) for PRT and its major metabolites following i.m. administration of PRT to camels at 5 mg/kg (n=6) and goats at 10 mg/kg (n=5).

	Camels			Goats		
Parameter	Paracetamol	β-Glucuronide	Sulphate	Paracetamol	β-Glucuronide	Sulphate
t_{max} (min)	23.33 ± 4.94	120.00 ± 7.75	76.67 ± 8.82	14.00 ± 4.00	36.00 ± 3.67	37.00 ± 5.15
C_{max} (μg/ml)	4.05 ± 0.74	7.03 ± 1.40	13.08 ± 2.51	4.67 ± 0.47	44.53 ± 5.60	1.84 ± 0.42
$AUC_{0\text{-}last}$ (μg.min/ml)	371.8 ± 49.8	1057.8 ± 194.0	1729.1 ± 301.7	198.7 ± 18.7	3856.1 ± 258.0	123.5 ± 35.0
MRT (min)	72.4 ± 3.0	114.5 ± 2.0	100.2 ± 3.2	36.2 ± 3.8	74.4 ± 4.8	58.7 ± 5.4
MAT (min)	26.1 ± 1.7			10.0 ± 2.2		
$F_{0\text{-}last}$ (%)	105 ± 26*			71 ± 17†		

* n = 5

† n = 4

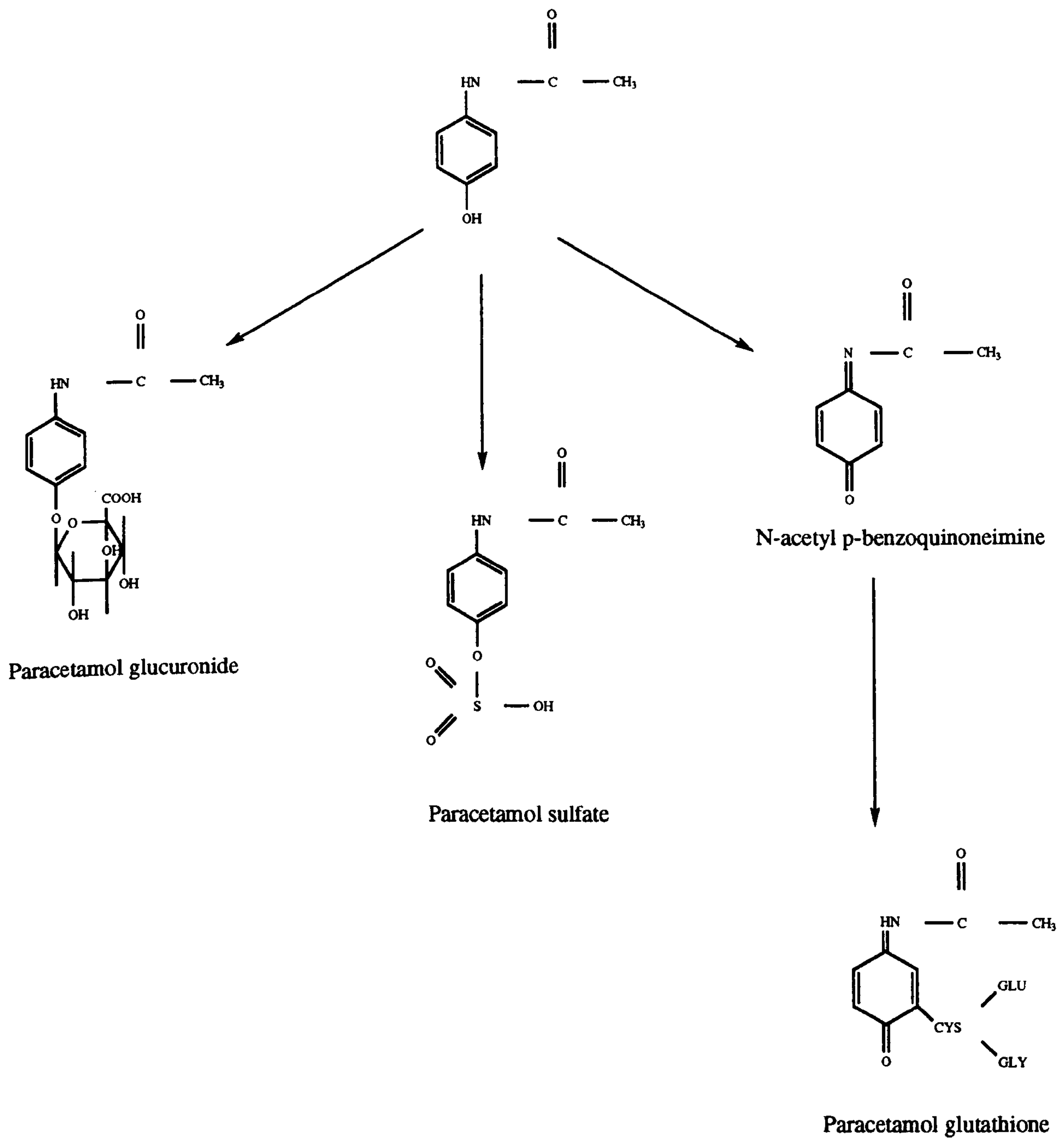


Fig.8.1. Major metabolic pathways of paracetamol

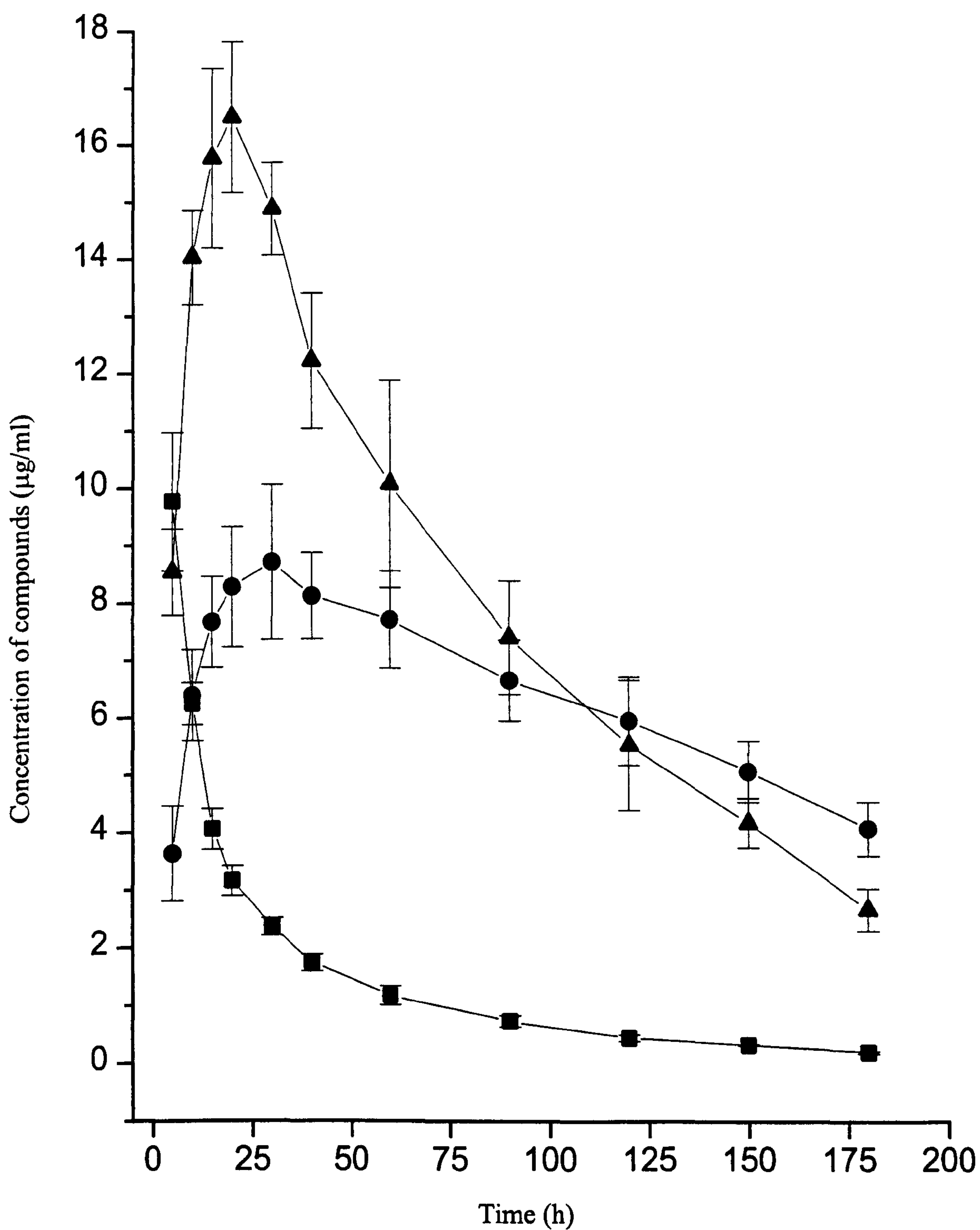


Fig. 8.2. Concentration- time curves of PRT (—■—), PRT glucuronide (—●—) and PRT sulfate (—▲—) in plasma following i.v. administration of PRT at a single dose rate of 5.0 mg/kg in the camels (n=6, mean ± SE).

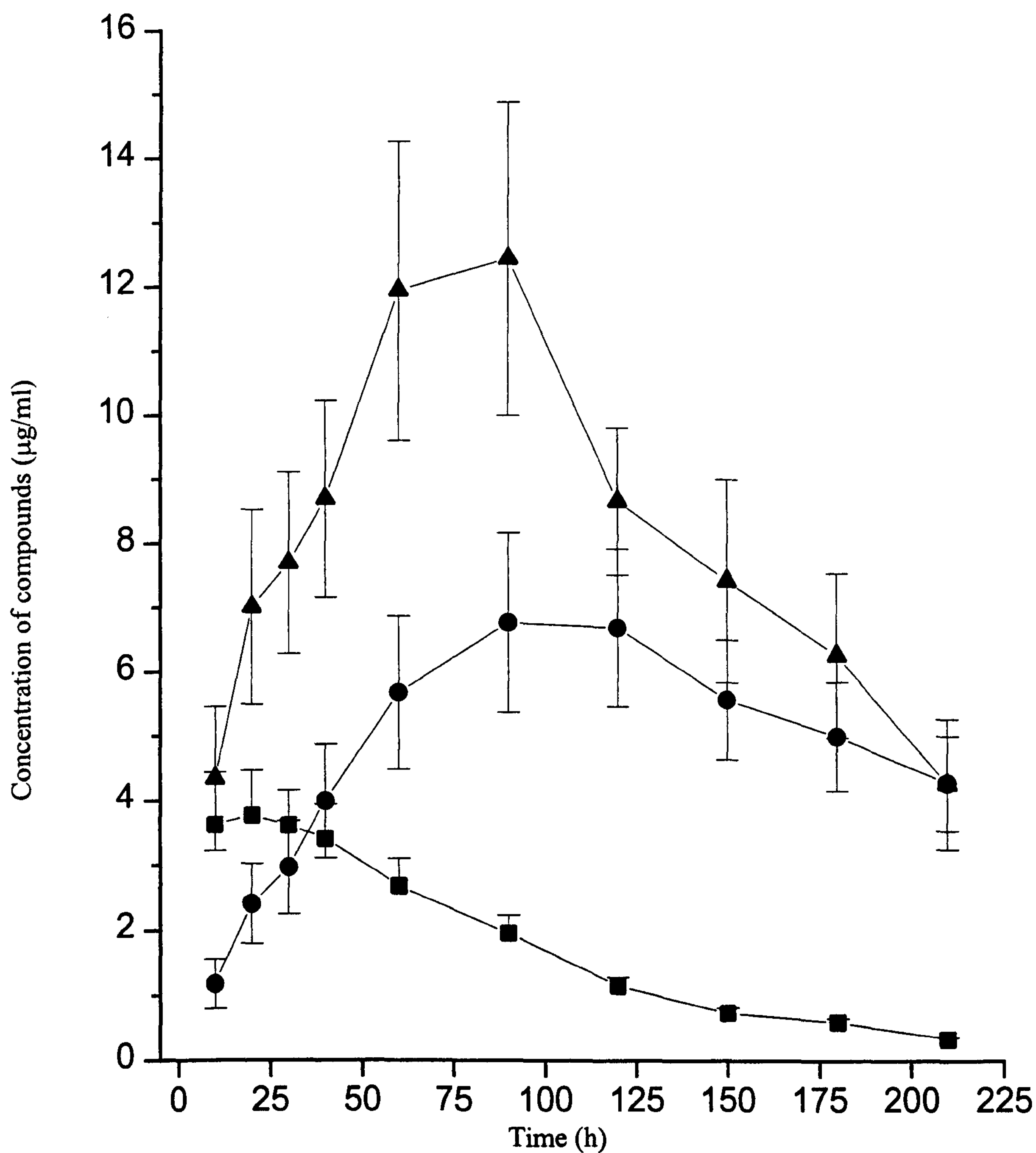


Fig. 8.3. Concentration versus time plot for PRT (—■—), PRT glucuronide (—●—) and PRT sulfate (—▲—) in plasma following i.m. administration of PRT at a single dose rate of 5.0 mg/kg in camels (n=6, mean ± SE).

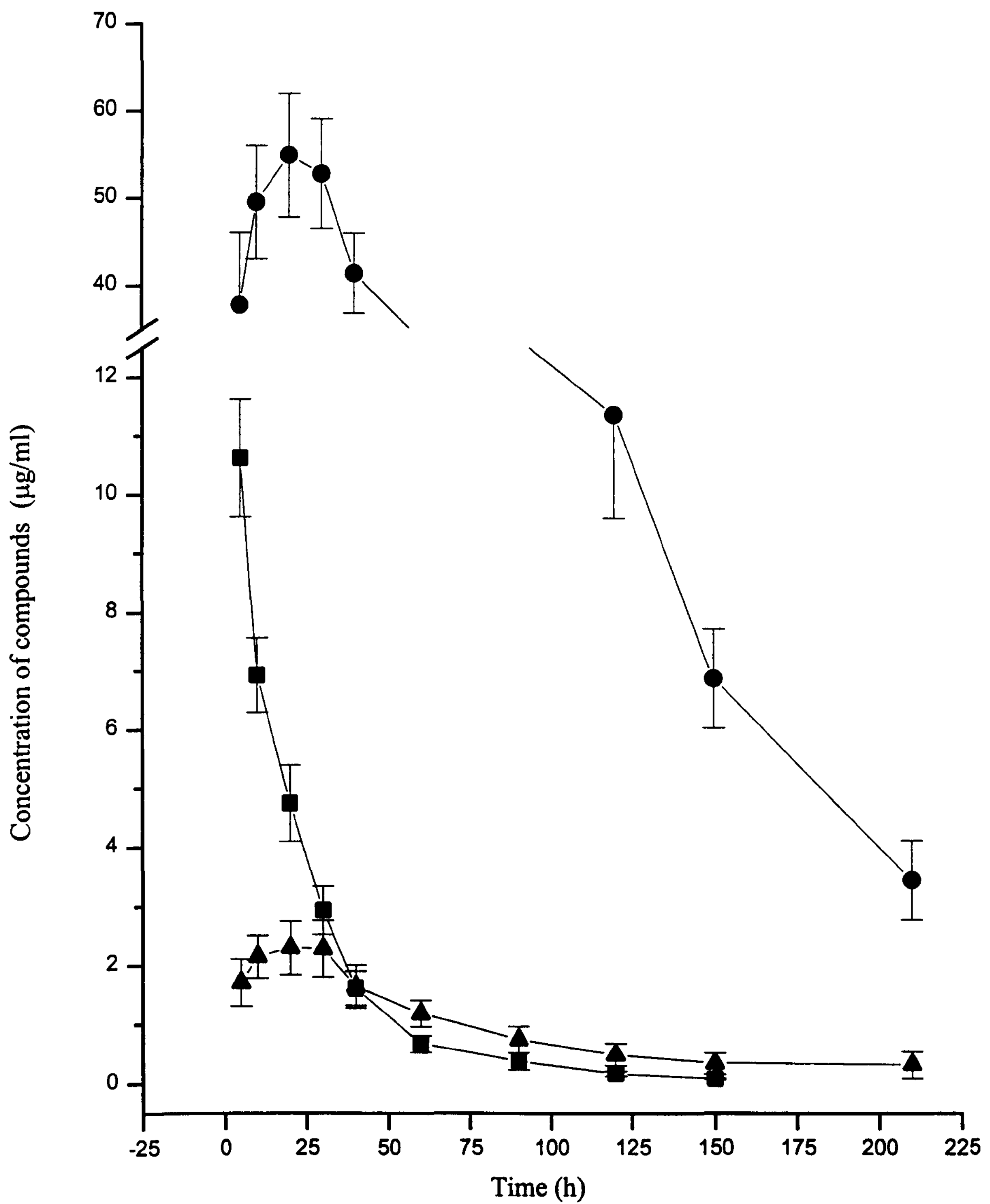


Fig. 8.4. Concentration versus time plot for PRT (—■—), PRT glucuronide (—●—) and PRT sulfate (—▲—) in plasma following i.v. administration of PRT at a single dose rate of 10 mg/kg in goats (n=6, mean ± SE).

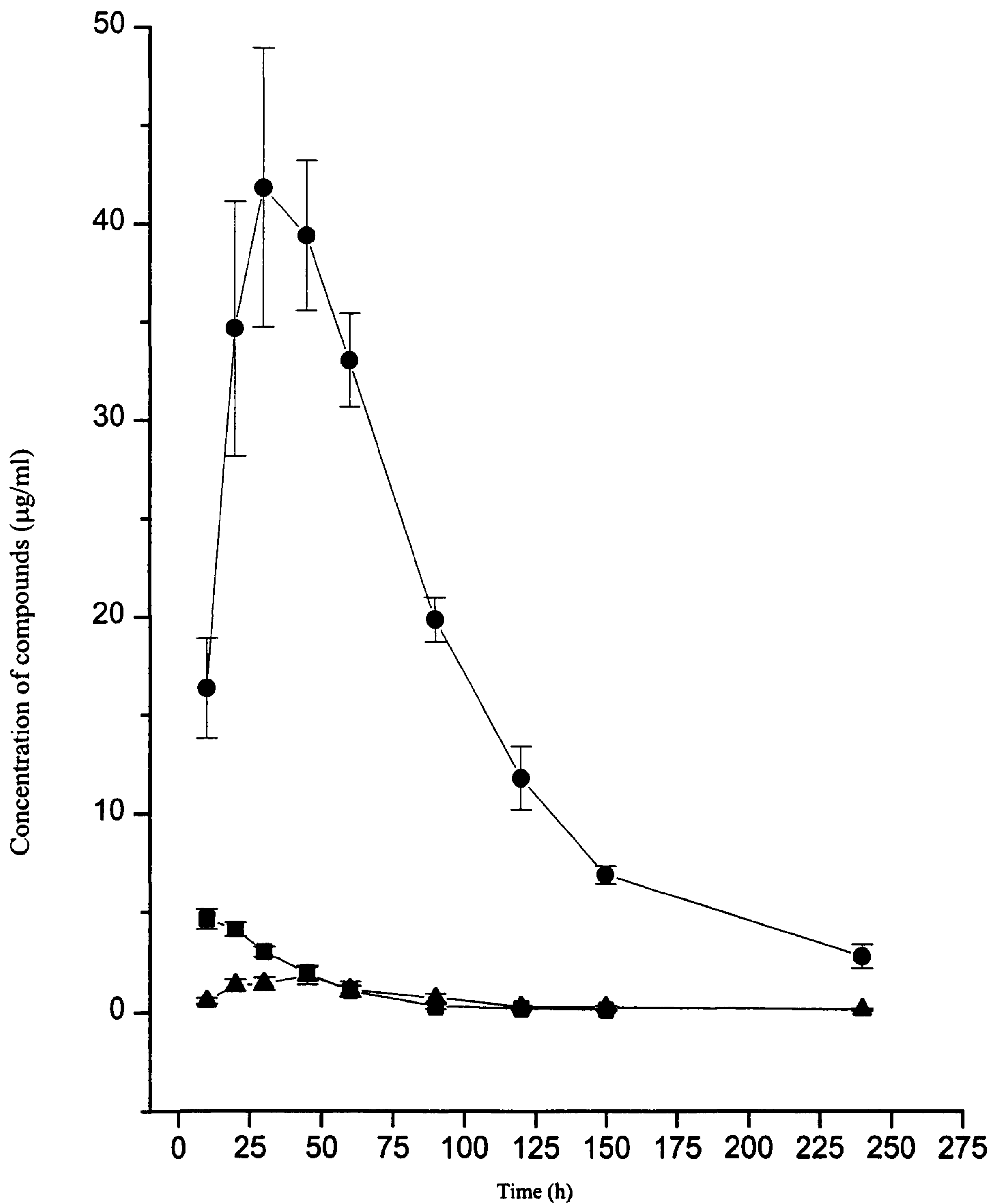


Fig. 8.5. Concentration versus time plot for PRT (—■—), PRT glucuronide (—●—) and PRT sulfate (—▲—) in plasma following i.m. administration of PRT at a single dose rate of 10 mg/kg in goats (n=6, mean ± SE).

Care must be exercised when comparing data from different animal species given different doses of PRT. In the present study PRT was administered at 5 mg/kg to camels and 10 mg/kg to goats and in the studies by Prescott (1980) and Greenblatt and Engelking (1988) it was administered at 12 mg/kg in man and approximately 9-15 mg/kg in ponies. Paracetamol metabolism has been shown to be both age (Levy *et al.*, 1975) and dose (Prescott, 1980) dependent and the dose may have affected the metabolism and elimination of PRT in the above studies. It is unlikely however that the dose affected the absolute comparisons made between camels and goats since the goats received the larger dose and had more rapid clearance, although these comparisons may have been affected quantitatively. All animals used in the present studies were healthy mature adults and the influence of immaturity of metabolising mechanisms was not a feature of the present study. The doses of PRT used in the present study were selected empirically, 10 mg/kg is within the dose range used in man and the lower dose of 5 mg/kg has been recommended for infants since infants have lower metabolising capacity. The lower dose used in the camel took cognisance of the known high susceptibility of the camel to drug toxicity (Ali, 1988). The lower dose used in camels was also chosen because solubility of PRT (pure compound) in saline was limited (to about 1 g/70 ml) and the dose volume required to administer 10 mg/kg would have been excessive.

Ruminants generally have more active glucuronidation and sulphation processes for xenobiotics than monogastric species (Smith *et al.*, 1984; Short *et al.*, 1988) and although there is relatively little information on metabolism in the camel, it has been shown to have lower ethoxycoumarin-o-deethylase and glutathione-s-transferase activity than sheep or goats (El Sheikh *et al.*, 1991). The activity of the glutathione-s-transferase could be of importance in PRT overdosage in the camel since glutathione conjugation is thought to be a limiting step in removal of intoxicating metabolites of PRT (Gregus *et al.*, 1988). Unfortunately it was beyond the scope of the present study to characterise the glutathione and hydrolysed glutathione products of PRT metabolism. Since these products are largely excreted in the bile of several species and very low plasma concentrations occur biliary cannulation would be required to accurately describe their generation (Gregus *et al.*, 1988).

In the present study PRT had a relatively large volume of distribution (1210 ± 42 ml/kg in camels and 1418 ± 138 ml/kg in goats) compared with other antipyretic analgesics in domestic

animals which generally have volumes of distribution of less than 300 ml/kg. The larger V_{ss} for PRT has also been reported in man [897 ± 94 ml/kg (Prescott, 1980)] and in ponies [2510 ml/kg (Greenblatt & Engelking, 1988)] and may be due to the more lipophilic, phenolic structure of PRT compared with the carboxylic acid structures of other NSAIDs. The larger volume of distribution of PRT may contribute to its known central analgesic and antipyretic activity by permitting a larger amount of drug to gain access to the site of action.

In the present study all animals had free access to water and were considered to be well hydrated at all times. Hydration is known to affect the V_{ss} of drugs in camels and the distribution volume of antipyrine has been shown to be doubled in dehydrated camels (Ben-Zvi *et al.*, 1994). In the same study oxidative metabolism was also reduced in dehydrated camels, and it may be important to assess the degree of hydration in camels before administration of potentially toxic drugs known to be metabolised by the liver.

The present study was carried out as a two phase study with i.m. administration ten days before i.v. administration in the same animals. The design has the drawback that period differences which could occur because of enzyme induction or other factors are not controlled. A more appropriate design where bioavailability is being examined incorporates a cross-over sequence such that half the animals receive the drug by each route on each occasion. While the experimental design used in the present study precludes an accurate determination of the absolute bioavailability of PRT given i.m. to camels or goats, it does suggest that rapid enzyme induction to PRT metabolism may occur in the camel. Since the ratio of glucuronide:PRT changed from 2.82 ± 0.31 following i.m. administration to 5.20 ± 0.50 following i.v. administration and there was a smaller change in ratios of sulphate : PRT it is possible that induction occurred in the glucuronidation pathway in the camel. The ratios of glucuronide : PRT and sulphate : PRT did not change significantly ($P > 0.05$) in goats between the phases of the study suggesting that induction did not take place in this species. It has been demonstrated that in man where first pass metabolism occurs following p.o. administration, the metabolic pathway may be dose-dependent and inducible (Rawlins *et al.*, 1977; Perucca & Richens, 1979).

In conclusion, the present study demonstrated large differences in PK and metabolism of PRT in camels and goats and confirms the danger of extrapolating dosages of potentially toxic drugs from man and laboratory animal species to ruminant species (van Miert 1989).

Chapter 9

General discussion

9.1. The acute inflammatory process and PK and PD of the tested NSAIDs

Higgins and Lees (1984a, 1984b, 1984c) developed a subcutaneous tissue-cage implantation equine model of acute inflammation for investigating the inflammatory process and PK and PD of NSAIDs. Following the successful application in equine species (Lees *et al.*, 1987c), it was soon used in other animal species, including dogs (McKellar *et al.*, 1994a, 1994b) and cattle (Landoni *et al.*, 1995a; Lees *et al.*, 1996). This model is now often referred to as the 'tissue-cage model' and has become a standard model for testing PK and PD of NSAIDs in veterinary science. In the present study, we adapted the model for sheep with some modification. In the equine and bovine, the model incorporated a tissue-cage comprising a ball with an internal volume of 31 ml and inflammation was initialised and maintained by intracaveal injection of 0.5 ml 1% carrageenan. In the ovine model, differences of body size between sheep and ponies or cattle led to the application of a smaller ball for implantation. The ball was sized 2.0 cm in diameter with an internal volume of 4.2 ml and two balls were implanted in each side of the neck of the sheep. The acute inflammation was stimulated by injection of 0.3 ml of 1 % carrageenan into the cages and maintained by a further injection of 0.2 ml carrageenan after 8 h. Following the injection of the mild irritant, carrageenan, the acute inflammatory processes developed well. About 1 ml of exudate and transudate could be consistently harvested from the target cages at 2 h intervals and this was sufficient for the analysis of inflammatory mediators and drug concentration. The concentration of exudate PGE_2 rose from an undetectable level to 35 ng/ml at 12 h and was accompanied with the increase in exudate LTB_4 generation and skin temperature over the cages. The concentrations of exudate PGE_2 in sheep was lower than the reported concentrations in the equine model (197.0 ± 64.9 ng/ml at 12 h) (Higgins & Lees, 1984a), but the infiltration of WBC in the present study (a peak number of 41.95 ± 8.95 × 10^9 cells/L at 24 h) was more intensive than in the equine model (peak value of 21.8 ± 4.1 cells/L at 12 h) (Higgins *et al.*, 1987).

The present study demonstrated that the changes in inflammatory processes were mild, reproducible, reversible and time-dependent, indicated by the changes in inflammatory mediators, cells and skin temperature over the cages. The sheep were kept after use and apparently recovered from the inflammatory lessons completely. The animals were used repeatedly and cross-over studies undertaken in an ethically acceptable way. In the present study, two experiments incorporated a total of 8 cross-overs over a 12 month period. No tissue-cages were damaged or became unusable over the period of use. Although there were differences of serum TXB_2 and PGE_2 generation between cross-overs, the changes occurred in all animals, were associated with age or environment and were parallel effectively controlled by the Latin square cross-over design and appropriate ANOVA.

In order to estimate the pain caused by intracaveal injection of carrageenan and needle puncture for sampling, the thresholds to noxious mechanical and thermal stimulation were measured using a method reported previously (Nolan *et al.,* 1987). The results showed that in the PLB-treated group there was no significant differences in mechanical and thermal thresholds between the control values (measurements before carrageenan injection) and the values after carrageenan injection and repeated sampling over a period of 2-96 h ($P>0.05$) (data are not reported in this thesis). This is the first time that pain has been investigated in the tissue-cage model and the findings indicate that pain is not intense even when the acute inflammatory process is well developed in the tissue-cages. This supports the original objectives outlined by Higgins and Lees (Higgins *et al.*, 1984; Higgins & Lees, 1984a, 1984c; Higgins *at al.,* 1987; Lees *et al.*, 1987c), in which their objectives were to develop an acute inflammatory model without causing distress to the animals. This also suggests that this model is not suitable for testing the analgesic properties of NSAIDs.

It is interesting to notice that the overall mean values of serum TXB_2 and exudate PGE_2 in the PLB-treated group in the second experiment (26.16 ± 0.62 and 35.35 ± 4.64 ng/ml, respectively) (reported in Chapter 4) were significantly higher ($P<0.01$) than in the first experiment (19.10 ± 10.20 and 16.32 ± 1.07 ng/ml, respectively) (reported in Chapter 3). The reason for this is not clear. It is possible that repeated injection of carrageenan facilitates the generation of exudate PGE_2 by increasing the extension of fibrovascular granulation tissue into the perforated cages. It has been reported that in the tissue-cage model, fibrovascular granulation tissue is subject to

stimulation of irritants (Higgins & Lees, 1984c; Higgins *et al.*, 1987). However this can not explain the increase in serum TXB_2 generation since this was generated *ex vivo* in clotting blood. It, therefore, is likely that the increase is by enzymatic induction since COX derived eicosanoids are involved in host defensive mechanisms and after inhibition by NSAIDs, the body may regulate its defensive function by enhancing the activity or quantity of COX isoenzymes. This explanation was supported by the results that the overall mean concentration of exudate LTB_4 in the second experiment (1.07 ± 0.07 ng/ml) was somewhat lower than in the first experiment (1.26 ± 0.09 ng/ml) and that 5-LOX was not inhibited by PBZ and FM. If this is true, it suggests that the body may develop "resistance" to inhibition of COX by repeated use of NSAIDs. It is also possible that the activity and quantity of COX in sheep is age-dependent since at the beginning of the first cross-over experiment the sheep were about one year old whereas they were about one and half years old at beginning of the second cross-over experiment.

The mechanisms of carrageenan-induced tissue-cage in inflammation were studied in the ponies (Higgins & Lees 1984a, 1984b, 1984c; Higgins *et al.*, 1987; Lees *at el.*, 1987c), dogs (McKellar *et al.*, 1994a, 1994b) and cattle (Landoni *et al.*, 1995a; Lees *et al.*, 1996). Following the implantation of tissue-cages, they became encased in fibrous tissue. Capillaries grew rapidly into the cavity through the perforation until a mass of fibrovascular granulation tissue filled the centre of the cages. The tissue in the cages was subject to carrageenan stimulation. Carrageenan is derived from the seaweed *Chondrus crispus*. The active fraction is a sulphated polymer of D-galactose bearing structural resemblance to chondroitin sulphate A. This provided a mild irritation which developed an acute inflammation.

Leukocyte infiltration into exudate occurred following intracaveal injection of carrageenan solution. The cells were predominately neutrophils and lymphocytes. Cell numbers rose at 4, 8, 12 and 24 h and decreased thereafter. The second injection of carrageenan at 8 h strengthened the recruitment of WBC ($18.33 \pm 4.10 \times 10^9$ cells/L at 8 h and $41.95 \pm 8.95 \times 10^9$ cells/L at 24 h). The maximal concentration of PGE_2 and LTB_4 in exudate occurred at 12 h which was 4 h following the second injection of carrageenan. This suggested that the dose rate of carrageenan used in the present study (0.3 ml for the first injection and 0.2 ml for the second injection) was appropriate since it caused acute and reversible inflammatory responses.

The present study showed that arachidonic acid metabolites play an important role in the acute inflammatory processes. Following the injection of carrageenan, the metabolism of arachidonic acid was extensive. Both COX and LOX metabolic pathways of arachidonic acid were activated, as indicated by the increased concentrations of PGE_2, LTB_4 and 12-HETE in the inflammatory exudate. The increase in exudate PGE_2 was correlated with an increase in skin temperature over the cages and the inhibition of exudate PGE_2 by PBZ, FM and CPF produced an inhibition of the temperature rise. For example, FM abolished exudate PGE_2 generation for up to 8 h and accordingly the increase in skin temperature over the cage was completely inhibited for up to 8 h (Chapter 3). Phenylbutazone attenuated exudate PGE_2 generation and simultaneously attenuated the increase in skin temperature. It is possible that COX derived eicosanoids, including PGs and TXs, accounted for the increase in skin temperature since PGs are powerful vasodilators which increase local blood flow and cause an increased temperature in the inflammatory sites. The increased temperature may also be augmented by increased energetic metabolism caused by the vasodilatation in the inflammatory sites. Previous studies of CPF in dogs (McKellar *et al.*, 1994a) showed that CPF did not inhibit exudate PGE_2 or skin temperature over the cages. The present studies supports the belief that PBZ and FM produce their therapeutic effects by inhibition of COX isoenzymes. However in the experiment reported in Chapter 4, the increase in skin temperature over the cage was not as high as in the experiment illustrated in Chapter 3 whereas the increase in exudate PGE_2 was more intensive. The inhibition of exudate PGE_2 by rac-CPF and S(+)CPF was not consistently correlated with the changes in temperature. The reason for this may be that following 3 cross-overs (Chapter 3), the tissue-cages became old and encased in more fibrous tissue and thus less responsive to the changes in inflammatory temperature. It is also possible that in addition to PGs, other factors contribute to the increased inflammatory temperature since inflammation is a very complicated process and a wide range of factors are involved. Previous studies on PBZ and FM in ponies (Lees *at al.,* 1987c) and tolfenamic acid in dogs (McKellar *et al.*, 1994b) showed that these NSAID produced more than 90 % inhibitory effect for exudate PGE_2 but that inhibition of skin temperature was moderate only.

It is believed that inhibition of COX by NSAIDs leads to metabolic diversion of arachidonic acid to increase LOX-derived eicosanoids, such as 12-HETE and LTB_4 (Higgs *et al.*, 1980; Higgins & Lees, 1984b; Sedgwick *et al.*, 1987). This may be considered as a side effect of NSAID treatment

since LTB_4 and 12-HETE are a powerful chemokinetic, chemotactic and chamoattractant agents for WBC (Ford-Hutchison *et al.,* 1980; Higgins & Lees, 1984b; Levine *et al.*, 1984; Lees *et al.*, 1987c; Sedgwick *et al.*, 1987; William, 1994). There is a general belief that NSAIDs relieve the symptoms of chronic inflammatory joint diseases while they do not retard the rate of development of the organic disease process and may even hasten the progress of disease (Tobin *et al.*, 1986). Metabolic diversion appeared to occur in the present study in sheep (Chapter 3 and 4). However the changes in exudate WBC recruitment were different between the tested NSAIDs. Phenylbutazone and FM treatments increased LTB_4 generation in exudate and this was accompanied by an attenuated exudate WBC accumulation, while rac-CPF and S(+)CPF treatments increased LTB_4 generation and WBC recruitment in exudate simultaneously. The mechanisms included in these changes are unknown. The findings with rac-CPF and S(+)CPF treatment were expected since increased LTB_4 would be expected to lead to an increase in WBC recruitment. The findings for the PBZ and FM treatments were unexpected and may be explained as follows: 1) Although PGE_2 has been considered as a weak chemokinetic and chemotactic agent *in vitro* (Kitchen, *et al.*, 1985; Sedgwick *et al.*, 1987), *in vivo* it is a powerful vasodilator. Increased local blood flow and vascular permeability may have facilitated infiltration of WBC into the inflammatory sites and thus the NSAIDs may downregulate WBC accumulation in inflammatory exudate by inhibiting exudate COX. 2) The NSAIDs may block leukocyte migration directly since PBZ and FM have been shown to inhibit zymosan-activated PMN and mononuclear leukocyte movement *in vitro* (Dawson *et al.*, 1987; Sedgwick *et al.*, 1987) and to inhibit LTB_4 - directed migration of canine PMN *in vitro* and *ex vivo.* 3) Recruitment of WBC to inflammatory sites is a complicated process and a number of chemical mediators are involved, including PAF, complements and adhesion molecules. Phenylbutazone and FM may affect some of these mediators and thus modify WBC recruitment in inflammatory sites.

Research has now identified two isoforms of COX existing in mammalian cells, named COX-1 and COX-2 (Kujubu *et al.,* 1991; Xie *et. al.*, 1991; O'Banion *et al.*, 1992; Sirois *et al.*, 1992; DeWitt & Meade, 1993; Jones *et al.*, 1993; Herschman, 1994). Cyclooxygenase-1 is a constitutive enzyme and generates a basal level of PGs which modulate physiological processes, such as homeostasis while COX-2 is an inducible enzyme and generates PGs during inflammation following stimulation. Prostaglandin E_2 has been shown to be the predominant PG present in acute inflammatory exudate in ponies (Higgins *et al.*, 1984; Higgins & Lees 1984a).

There are several reasons why the exudate PGE_2 in the present study was predominantly generated by COX-2. Firstly, COX-2 is an inducible enzyme which does not express unless stimulated (Williams & DuBois, 1996). In the present study, exudate PGE_2 was undetectable prior to carrageenan injection and it increased sharply following carrageenan injection. Secondly, the concentration time course of exudate PGE_2 generation showed that exudate PGE_2 was associated with COX-2. It has been demonstrated using molecular biological methods that COX-1 and COX-2 associated PGD_2 generation occurs in two temporally distinct periods in activated mast cells. The first phase, catalysed by COX-1, was completed in 30 min. This was followed by a 15-fold induction of steady-state transcripts of COX-2 and reached a peak at about 6 h while the expression of COX-1 did not change during this period (Kawada *et al.*, 1995; Murakami *et al.*, 1995). The present study and other studies (Higgins *et al.*, 1984; Higgins & Less, 1984a; McKellar *et al.*, 1994a, 1994b; Landoni *et al.*, 1995a) showed that PGE_2 concentration in exudate increased markedly to a maximum which occurred at 6-12 h following injection of carrageenan. This corresponds with the above *in vitro* findings. Thirdly, COX-1 and COX-2 isoenzymes are pharmacologically distinct for NSAIDs (Meade *et al.*, 1993; Herschman, 1994; Laneuville *et al.*, 1994; Johnson *et al.*, 1995; Williams & DuBois, 1996). In the present study in Chapter 3, PBZ and FM produced differential inhibitory effects on carrageenan-induced exudate PGE_2 and PLT COX (COX-1). The selective inhibition of PBZ for serum TXB_2 and exudate PGE_2 in the present experiment was in accordance with the results of *in vitro* experiments using COX-1 and COX-2 cDNAs (Laneuville *et al.*, 1994). Finally, in a study using a rat carrageenan subcutaneous air pouch model, the selective COX-2 inhibitor (NS-398) significantly reduced exudate PGE_2 generation and subsequent inflammation (Masferrer *et al.*, 1994). In the present study we simultaneously tested the inhibitory effects of the NSAIDs on serum TXB_2. It has been shown that serum TXB_2 was predominantly generated by COX-1 in PLT (Funk *et al.*, 1991). Cyclooxygenase-2 is thought to be the therapeutic target for NSAIDs and inhibition of COX-2 by NSAIDs generates antipyretic, analgesic and antiinflammatory action but the simultaneous inhibition of COX-1 results in unwanted side effects, particularly those leading to gastric ulcers (Herschman, 1994; Williams and DuBois, 1996). Therefore the inhibitory selectivity for serum TXB_2 and exudate PGE_2 is a convenient and important criterion used for testing the therapeutic and side effects of NSAIDs *in vivo*.

The results in Chapter 3 and 4 showed that following two injections of carrageenan solution into the tissue-cages, concentrations of PGE_2 increased in a time-related fashion. Exudate PGE_2 concentration decreased sharply after 32 h in both cross-over experiments and inter-animal differences were very large (Co-Var > 93 % in Chapter 3 and > 99 % in Chapter 4). The results in Chapter 4 showed that exudate PGE_2 generation was not significant after 32 h compared with the pre-values although the exudate PGE_2 was detectable. This indicates that from 32 h after carrageenan injection the model was less reliable for evaluating COX inhibitors. This may explain the fact that in the experiments in sheep FM, rac-CPF and S(+)CPF all produced significant inhibitory effect for exudate PGE_2 from 4 to 32 h although FM inhibited exudate PGE_2 generation by more than 50 % up to 144 h and the concentration of exudate PGE_2 in the rac-CPF and S(+)CPF groups were lower than that in the PLB group from 32 h to 144 h. For some of the NSAIDs with slow elimination rates the drug concentrations were high at or after 32 h following drug administration. This suggests that a third or fourth injection of carrageenan into the tissue-cage may be required to achieve reliable information regarding COX inhibition in inflammatory exudate after administration of NSAIDs at certain dose rates. However care should be taken since it has not been determined whether the inflammatory responses in the tissue-cage are still reversible following more than two repetitions of carrageenan injection. It is possible that an appropriate reversible inflammatory response may be achieved by adjusting the dose rate of carrageenan for the repeat administrations. However, long term stimulation may lead to a chronic inflammatory lesion.

The present study showed that NO plays a role in the acute inflammatory process in sheep. Increased NO concentration in exudate was correlated with the generation of PGE_2 and LTB_4 in exudate. Nitric oxide is believed to be a vasodilator and to play a role in inflammation (Moncada *et al.*, 1991, Wei *et al.*, 1995). The present study agrees with the finding that COX isoenzymes are potential receptor targets for NO (Salvemini *et al.*, 1994) and also suggests that NO stimulates the activity of 5-LOX. Therefore NO may serve as a pro-inflammatory mediator in the tissue-cage inflammatory model in sheep and it is possible that a combination of COX-2 and iNOS inhibitors will provide wider antiinflammatory effects than NSAIDs alone.

As reported and discussed in Chapters 3, 4 and 6, the inhibition of PLT COX (COX-1) and exudate COX (COX-2) by PBZ, rac-CPF and S(+)CPF was different in each target species. The

mechanism for this finding is not clear. It is unlikely that the differences resulted from differences in drug concentration in plasma and exudate since C_{max} values of PBZ in the sheep were higher than in horses and donkeys. For example, C_{max} of PBZ in exudate was 22.32 μg/ml in sheep, 12.40 μg/ml in horses and 1.30 μg/ml in donkeys whereas PBZ inhibited exudate PGE_2 effectively in the horses (Lees *et al.*, 1987c) and donkeys (Chapter 6) but not in sheep (see Chapter 3). It is possible that the gene sequences of COX isoenzymes are different between animal species. It is also possible that the concentrations of peroxide in exudate and serum are different between animal species since peroxides interrupt the inhibitory effects of NSAIDs, especially the weak inhibitors of COX (Marshall *et al.*, 1987; Hanel & Lands, 1982). This suggests that the PD data for some NSAIDs can not be extrapolated between animal species. However FM did not show species differences for the inhibitory effect on the COX enzyme. It abolished or effectively inhibited serum TXB_2 and exudate PGE_2 at a dose rate of $\geq$ 1.1 mg/kg, in all tested animal species including horses (1.1 mg/kg) (Lees & Higgins 1984; Lees *et al.*, 1987a, 1987c), calves at 1.1 - 6.6 mg/kg by Lees *et al.* (1991c) and at 2.2 mg/kg by Landoni *et al.* (1995a) and dogs (0.55-1.65 mg/kg) (McKellar *et al.*, 1989).

Inhibition of WBCs and PLT in venous blood may be associated with some side effects of NSAIDs since such inhibition is associated with immuno-suppression and blood dyscrasias. The present studies demonstrated that none of the tested NSAIDs modified the numbers of WBC and PLT in venous blood in the tested animal species. The NSAIDs in the present study were given as a single dose and the dose rates were determined following clinical recommendations in horses (PBZ and FM) or dogs (CPF). Thrombocytopaenia and leukopaenia may occur during the treatment with NSAIDs, especially PBZ in horses (Lees & Higgins, 1985). However this happens only at high dose rates or following repeated administration for a long duration.

In the present study, the PK of PBZ was investigated in sheep, goats and donkeys and the results showed inter-species differences and similarities. The elimination of PBZ in plasma (Chapter 6), exudate and transudate in the donkey study was faster than in horses (Piperno *et al.*, 1968; Lees *et al.*, 1986; Maitho *et al.*, 1986; Lees *et al.*, 1987c). The elimination of plasma PBZ in sheep was similar to the values in goats but it was faster than in the large ruminant species, such as cattle (Lees *et al.*, 1988). This may reflect a difference of hydroxylation function between animal species since the hydroxylated metabolite of PBZ, OPBZ in donkeys was more predominant than

in the horse and the ratios of OPBZ to PBZ in both goats (Chapter 7) and sheep (Chapter 3) were very low (<5 %).

In the present study the plasma kinetics of FM demonstrated less inter-species differences compared with PBZ. The elimination of FM from plasma in the donkeys ($t_{\frac{1}{2}\beta} = 2.09 \pm 0.45$ h, Chapter 7) was similar to that in horses ($t_{\frac{1}{2}\beta} = 1.94 \pm 0.24$ h, Lees *et al.*, 1987a, 1987c) and was also similar to that in sheep (2.33 ± 0.18 h). These values were shorter than the values reported in calves but the dose rate used in calves (Landoni *et al.*, 1995a) was double that used in sheep.

Because of the physicochemical properties including weak acid nature and high protein binding, NSAIDs accumulate in inflammatory sites so that the elimination of NSAIDs in exudate is slower than in plasma and transudate. This has, at least, been shown for FM in horses and calves (Lees & Higgins, 1984; Lees, 1989; Landoni *et al.*, 1995a), PBZ in horses (Lees & Higgins, 1986; Lees *et al.*, 1986), meloxicam in horses (Lees *et al.*, 1991b), CPF in dogs and calves (McKellar *et al.*, 1994a; Lees *et al.*, 1996), KPF in calves (Landoni *et al.*, 1995b) and tolfenamic acid in calves (Landoni *et al.*, 1996). In the present study, the elimination of PBZ and FM in exudate and transudate was slower than in plasma in all tested animals but the greater penetration in exudate than in transudate was achieved only for PBZ in donkeys. This may be explained by the intensive extravascular penetration for the NSAIDs in sheep. Flunixin meglumine and PBZ achieved a higher concentrations in exudate and transudate in sheep compared with the results reported in horses (Lees *et al.*, 1986) and calves (Landoni *et al.*, 1995a). This may also reflect a lower protein binding of the NSAIDs in sheep. The slower elimination of NSAIDs in exudate than in plasma may explain why NSAIDs often exert longer antiinflammatory effects than their plasma concentration would indicate. This may be of therapeutic interest since it confers more opportunity on the NSAIDs to produce their therapeutic effects. When determining the dose rates of a NSAID, particular attention should be paid to its kinetic behaviour in inflammatory sites.

The percentage inhibitory effect for serum TXB_2 produced by PBZ and FM declined in a sigmoidal fashion while the drug concentrations in plasma decayed exponentially (Fig. 9.1.). Thus it is difficult to predict the pharmacological responses from PK parameters. Pharmacokinetic/PD modelling has been widely used to establish the relationship between drug effect and concentration. When the pharmacological effects are associated with the plasma

(central compartment) or some other pharmacokinetically identifiable compartment, the effect-drug concentration data can be directly fitted to a PD model, such as sigmoid effect model (Gibaldi & Perrier, 1982d) and this may generate reliable results. If the pharmacological effects take place in other sites where the determination of the drug concentration is not possible, a mamillary effect compartment model may be used. This model assumes that the effect compartment is a separate compartment linked to the plasma compartment by a first-order process and receives a negligible mass of drug (Sheiner *et al.*, 1979; Whitting *et al.*, 1980; Gibaldi & Perrier, 1982d). Recently effect compartment models have been used for NSAIDs to establish the relationship between the antiinflammatory effects and the drug concentrations (Toutain *et al.*, 1994; Landoni *et al.*, 1995a, 1995b; 1996). Since it predicts PK in the effect compartment using plasma drug kinetics and for most of the NSAIDs the plasma kinetics are different from the exudate kinetics, the parameters generated may be somewhat misleading. In the present study, the effect of the NSAIDs on serum TXB_2 is associated with the plasma or central compartment and the effect on PGE_2 takes place in the tissue-cage exudate so that both pharmacological responses and drug concentrations are measurable. However, some NSAIDs, especially FM, has a longer inhibitory effects on exudate PGE_2 and serum TXB_2 than detectable drug concentrations would predict. For example FM was not detectable in plasma after 24 h in most animals while it produced 37.33 % inhibitory effect for serum TXB_2 generation in the sheep (Chapter 3). Pharmacodynamic fitting using drug effect against drug concentration is not applicable. In order to link drug effect and concentration, we applied the methods described in Chapter 2. The results showed that this method was practicable and reliable because it used drug effect and concentration directly determined in the associated sites or compartment. The elimination of PBZ in sheep was very slow so that the drug concentrations were detectable over a period of 144 h in plasma and exudate. We, therefore, used two methods to obtain the PD parameters for this drug. Firstly a Sigmoid inhibitory effect model using drug effect-concentration data was directly fitted and secondly the method described in Chapter 2 to link PK and PD was used. The results showed that both methods generated similar PD parameters. For example, in the estimation of the relationship between inhibitory effect of PBZ on serum TXB_2 and the drug concentration, the former method generated an E_{max} of 81 % and an IC_{50} of 9.98 μg/ml while the latter method produced an E_{max} of 80 % and an IC_{50} of 10.10 μg/ml. These methods, therefore, appear to be reliable for PK/PD modelling when the drug concentration and effects are identifiable.

9.2. Potential use of PBZ, FM and CPF in sheep

The present study in sheep showed that PBZ inhibits COX-1 and has a very weak inhibitory effect for COX-2. It did not significantly inhibit skin temperature over the cages. This suggests that PBZ has poor antiinflammatory and antipyretic effects in sheep. It may possess side effect, such as ulceration and loss of protein associated with COX-1 inhibition if it is used for a long duration although further confirmation of tolerance is required in sheep. It has been reported that the toxicity of PBZ is different between species, it has narrow therapeutic index in the horse but is relatively safe in the dogs (Tobin *et al.*, 1986; Lees, 1992). The PK study in sheep showed that the elimination of PBZ from plasma, exudate and transudate was very slow but that accumulation of the drug in the inflammatory site was not significant. Compartmental modelling showed PBZ had similar values for elimination half life in plasma ($t_{\frac{1}{2}\beta}$ = 17.92 ± 1.74 h), exudate ($t_{\frac{1}{2}K10}$ = 17.82 ± 1.27 h) and transudate ($t_{\frac{1}{2}K10}$ = 16.24 ± 1.60 h), and the concentration time curves confirmed that PBZ declined at same rates in plasma, exudate and transudate (Fig. 3.2.). The predicted residue times may be very long for PBZ in sheep since at 144 h after drug administration PBZ was detectable in all body fluids tested at relatively high concentrations (> 0.2 μg/ml). If the PBZ concentration in plasma following i.v. administration of PBZ at 4.4 mg/kg is extrapolated using the three compartmental model described in Table 3.2., the concentration would be 0.0053 ± 0.0023 μg/ml at 10 days and 0.00022 ± 0.0001 μg/ml at 14 days. Sheep are important food producing animals. If PBZ is used in this species for the purpose of food provision, the withdrawal periods may be very long. Therefore PBZ can not be recommended for clinical use in sheep based on the present studies.

Flunixin meglumine inhibits COX-1 and COX-2 intensely with a modest selectivity for COX-2 in the inflammatory exudate in sheep. The IC_{50} for COX-2 was very low and it produced 50 % of inhibitory effect for longer than 144 h following an i.v. administration at a single dose rate of 1.1 mg/kg in this species. It also abolished or significantly inhibited the increase in skin temperature over the inflammatory foci. The PK properties of FM are also attractive. It steadily penetrated into and was slowly eliminated from the inflammatory exudate so that the elimination from exudate was slower than from plasma. The drug became undetectable (below 0.05 μg/ml) at 24 h in plasma and at 72 h in exudate and transudate. Extrapolation based on the compartmental

model equation described in Chapter 3 predicted that at 144 h, the drug concentration would be 3.67×10^{-15} μg/ml in plasma, 0.00019 μg/ml in exudate and 0.00093 μg/ml in transudate. This suggests that a short residue time and withdrawal time may be appropriate in sheep. It has been demonstrated that FM had the greatest antiinflammatory and analgesic effects in horse among a group of NSAIDs, including meclofenamic acid, PBZ, naproxen and salicylate (Deegen, 1992) and that FM is relatively safe in horses (Lees & Higgins, 1985). Flunixin meglumine has been licensed in cattle in UK at 2.2 mg/kg, for administration i.v. once daily for up to 5 days and it has been widely used for the treatment of acute inflammation and other diseases including respiratory disease, fog fever, gastroenteritis, mastitis, endoxemia, anaphylactic reactions, arthropathies, bacterial and viral pneumonia (Espinasse, 1992). It is likely that FM is a useful NSAID for clinical use in sheep although tolerance studies are required before it can be recommended. Since the present study showed that an i.v. dose rate of 1.1 mg/kg was sufficient to abolish or inhibit COX-1 for up to 32 h and to produce over 50 % of inhibition for COX-2 for up to 144 h, it appears that the dose rate should be lower than 1.1 mg/kg if FM is repeatedly used in sheep.

In the present study in sheep CPF non-selectively inhibited COX-1 and COX-2 at an i.v. dose rate of 4.0 mg/kg and simultaneously inhibited skin temperature rise over the inflammatory sites. Further studies in our laboratory showed that CPF was an effective analgesic in sheep and that the analgesic effect was equal to FM (Welsh & Nolan, 1994b, 1995). Also in a study in dogs, CPF provided profound analgesia which was as effective and of longer duration than that produced by papaveretum, and was associated with significantly less postoperative sedation and a quicker return to the normal conscious state (Nolan & Reid, 1993). However at 4.0 mg/kg CPF did not significantly inhibit COX-1 or COX-2 in dogs (McKellar *et al.*, 1994a). Carprofen may be considered as a conventional NSAID with potent antiinflammatory and analgesic effects in sheep. However, the elimination of CPF in sheep was very slow. Following CPF administration iv at 0.7 and 4.0 mg/kg in the sheep, plasma $t_{\frac{1}{2}\beta}$ was 26.1 h and 33.7 h, respectively (Welsh *et al.*, 1992). In the present study rac-CPF was detectable at relative high plasma drug concentrations (>1.0 μg/ml) at 144 h after i.v. administration of rac-CPF at 4.0 mg/kg in sheep (Data not reported). This suggests that the withdrawal period of CPF may be long when used in sheep for food provision. Slower elimination of R(-)CPF than S(+)CPF has been shown in horses (Graser *et al.*, 1991; Lees *et al.*, 1991a), dogs (McKellar *et al.*, 1994a), calves (Lees *et. al.*, 1996)

and sheep in the present study (Data not reported). The S(+)CPF enantiomers contributes to the therapeutic effects and the R(-)CPF enantiomers may have side effects with less or no therapeutic effects. It may be desirable to develop a preparation containing the S(+)CPF enantiomer only for veterinary clinical use.

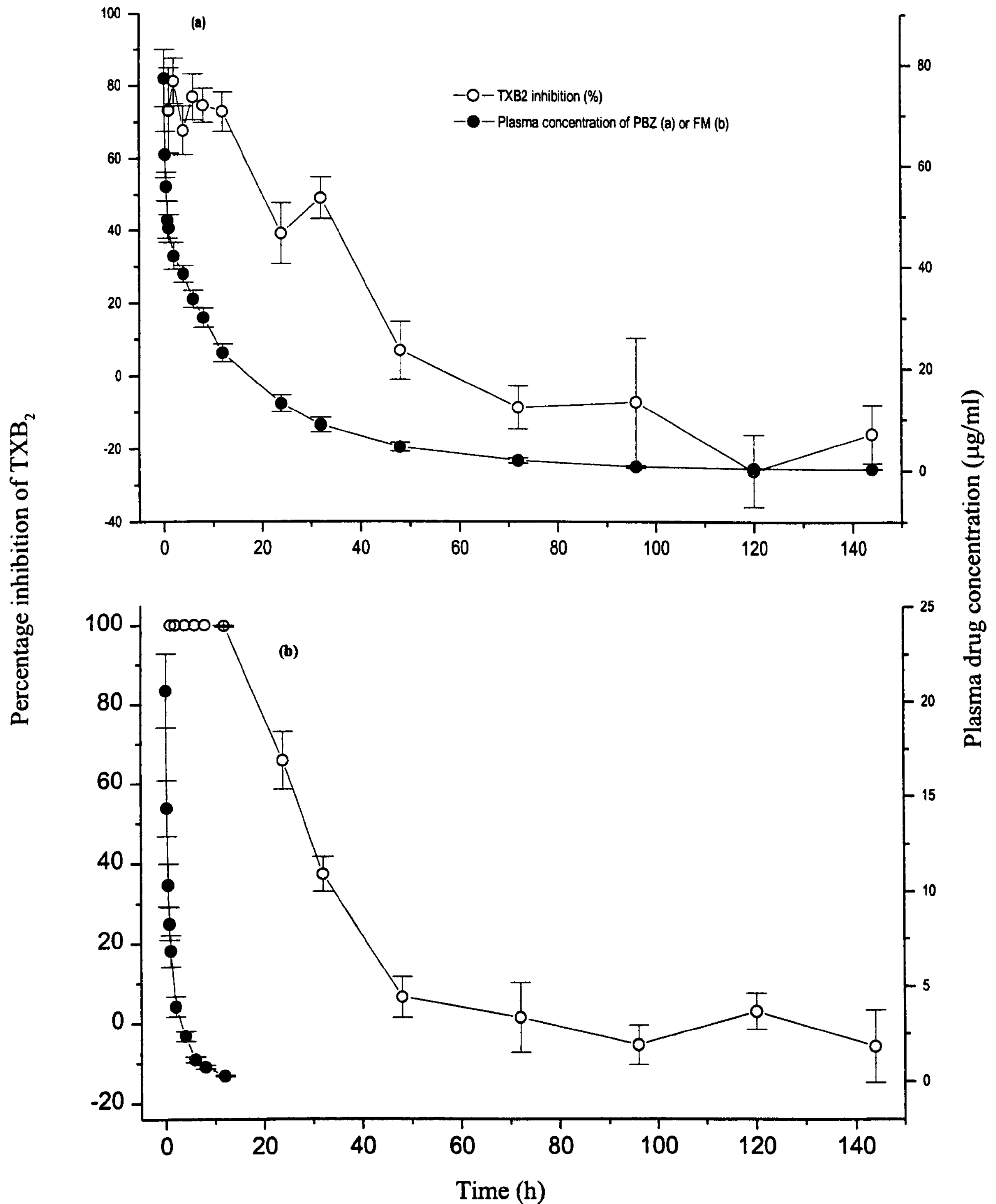


Fig. 9.1. Relationship between percentage serumTXB_2 inhibition and plasma drug concentration following PBZ (a), (4.4 mg/kg) and FM (b), (1.1 mg/kg) administered i.v. in 8 sheep (mean ± SE). Both PBZ and FM inhibit serum TXB_2 generation for up to 32 h ($P<0.05$).

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Appendix A

Pharmacokinetics and pharmacodynamics of phenylbutazone and flunixin meglumine in sheep

Experimental code: QM/ZC/11/94

Table A-1.
The concentrations (μg/ml) of phenylbutazone (PBZ) in plasma following intravenous (i.v.) administration of PBZ at 4.4 mg/kg in sheep.

	Animal numbers							
Time (h)	143	144	145	146	147	148	149	150
0.08	89.42	39.55	74.08	81.67	86.60	80.85	78.91	86.34
0.25	76.99	36.79	52.81	66.92	76.02	65.38	58.93	63.45
0.50	64.90	41.12	59.51	61.16	63.11	52.98	50.63	53.08
0.75	59.29	35.40	38.17	46.78	59.79	51.29	49.01	53.62
1.00	59.15	38.79	37.76	50.38	56.62	44.56	44.37	48.64
2.00	54.08	40.53	27.88	44.65	47.01	38.57	43.28	40.77
4.00	45.86	41.18	31.16	40.49	39.59	40.34	35.30	34.53
6.00	40.89	37.85	25.01	35.36	32.75	32.79	32.45	31.74
8.00	37.89	31.55	23.59	34.26	28.01	28.77	29.90g	25.35
12.00	32.54	19.06	16.40	24.14	23.75	23.61	25.26	19.22
24.00	22.06	8.88	7.67	14.39	12.42	15.29	15.12	8.49
32.00	14.05	6.32	4.55	12.57	6.90	11.61	8.80	5.91
48.00	7.71	2.99	1.56	7.33	2.51	6.20	5.50	2.34
72.00	3.90	0.77	0.34	3.39	1.00	3.13	2.36	0.55
96.00	1.66	0.22	0.09	1.39	0.25	1.21	1.11	0.15
120.00	0.90	0.25	0.02	0.34	0.11	0.57	0.53	0.69
144.00	0.49	0.09	0.00	0.73	0.07	0.31	0.22	0.00

Table A-2.
The concentrations (μg/ml) of phenylbutazone (PBZ) in exudate following intravenous (i.v.) administration of PBZ at 4.4 mg/kg in sheep.

	Animal numbers							
Time (h)	143	144	145	146	147	148	149	150
2	0.86	1.21	4.75	2.26	7.30	2.70	20.35	3.34
4	9.18	10.09	11.09	18.19	19.26	15.57	19.97	19.14
8	16.85	18.82	17.10	20.64	25.87	21.71	28.11	20.65
12	23.71	18.13	12.24	14.59	19.81	23.18	27.42	21.16
24	16.32	14.97	8.94	16.17	14.09	13.80	19.31	18.26
32	13.93	10.13	3.65	9.69	7.60	14.37	16.88	8.44
48	3.74	5.13	2.60	6.73	3.89	4.03	8.24	5.05
72	4.84	2.00	0.78	2.23	1.30	5.77	5.44	0.76
96	2.85	0.72	0.22	1.50	1.69	2.22	2.06	0.65
120	1.72	0.26	0.11	0.58	1.26	1.13	0.17	0.29
144	UD	UD	0.07	0.24	missing	0.34	misssing	0.12

UD = undetectable (<0.05 μg/ml)

Table A-3.
The concentrations (μg/ml) of phenylbutazone (PBZ) in transudate following intravenous administration of PBZ at 4.4 mg/kg in sheep.

	Animal numbers							
Time (h)	143	144	145	146	147	148	149	150
2	0.52	2.86	1.87	0.49	2.12	2.10	2.49	2.61
4	12.14	9.51	8.19	2.70	16.41	6.00	missing	21.23
8	22.41	15.80	19.08	14.61	24.23	11.06	21.17	26.06
12	27.69	20.12	18.35	missing	21.52	13.88	21.16	22.17
24	20.21	11.86	11.25	24.35	5.78	13.63	14.64	12.64
32	13.39	5.78	5.35	15.97	6.47	10.04	13.20	7.46
48	3.51	4.02	2.64	12.47	8.30	missing	5.77	3.17
72	3.43	1.29	1.00	6.66	2.09	2.44	3.00	1.23
96	3.51	0.31	0.04	3.20	missing	1.48	1.50	0.26
120	0.11	0.11	0.02	0.95	0.18	0.84	0.23	0.10
144	missing	0.10	missing	0.83	0.45	0.50	missing	missing

Table A-4.
The concentrations (μg/ml) of oxyphenbutazone in plasma following intravenous administration of phenylbutazone at 4.4 mg/kg in sheep.

	Animal numbers							
Time (h)	143	144	145	146	147	148	149	150
0.08	0.40	0.16	0.51	0.35	0.45	0.24	0.34	0.58
0.25	0.58	0.29	1.24	0.61	0.84	0.41	0.69	0.95
0.50	0.74	0.61	0.91	0.83	1.05	0.46	0.65	1.20
0.75	0.83	0.65	1.04	0.81	1.15	0.52	0.03	1.43
1.00	0.85	0.82	1.03	0.96	1.11	0.54	0.84	1.39
2.00	0.92	1.00	0.73	1.05	1.29	0.53	1.07	1.10
4.00	0.89	1.68	1.30	1.34	1.12	0.53	1.07	1.85
6.00	1.05	1.53	1.13	1.23	1.02	0.51	1.08	1.79
8.00	1.10	1.16	1.12	1.19	0.11	0.47	missing	1.28
12.00	0.93	0.77	0.74	0.99	0.84	0.31	1.06	1.26
24.00	0.68	0.48	0.63	0.71	0.73	0.38	0.76	0.71
32.00	0.55	0.33	0.44	0.54	0.45	0.31	UD	0.45
48.00	0.25	0.21	0.16	0.24	1.93	0.13	0.30	0.18
72.00	0.12	0.07	0.06	0.11	0.07	0.09	0.10	0.06
96.00	0.06	0.04	0.02	0.08	UD	0.06	0.07	UD
120.00	0.07	UD	UD	UD	UD	UD	0.05	UD
144.00	UD	UD	UD	UD	UD	UN	UD	UD

UD = undetectable (<0.05 μg/ml)

Table A-5.
The concentrations (μg/ml) of oxyphenbutazone in exudate following intravenous administration of phenylbutazone at 4.4 mg/kg in sheep.

	Animal numbers							
Time (h)	143	144	145	146	147	148	149	150
2	UD	UD	0.21	0.05	0.12	UD	0.37	0.06
4	0.16	0.29	0.54	0.53	0.59	0.16	0.37	0.68
8	0.35	0.67	0.81	0.64	0.83	0.24	0.28	1.30
12	0.54	0.42	0.65	0.54	0.74	0.25	0.76	0.61
24	0.50	0.66	0.52	0.80	0.61	0.24	0.55	1.40
32	0.41	0.42	0.13	0.51	0.48	0.24	0.48	0.43
48	1.53	0.24	0.24	0.29	0.27	0.23	0.28	0.15
72	0.17	0.12	0.05	0.10	0.05	0.09	0.09	0.12
96	0.07	0.04	UD	0.08	UD	0.05	0.07	UD
120	0.06	UD	UD	UD	UD	UD	UD	0.06
144	0.35	UD	UD	UD	missing	UD	missing	0.08

UD = undetectable (<0.05 μg/ml)

Table A-6.
The concentrations (μg/ml) of oxyphenbutazone in transudate following intravenous administration of phenylbutazone at 4.4 mg/kg in sheep.

	Animal numbers							
Time (h)	143	144	145	146	147	148	149	150
2	0.06	0.08	0.07	0.07	0.05	UD	0.05	UD
4	0.19	0.27	0.31	0.09	0.45	0.06	0.06	0.74
8	0.48	0.81	0.80	0.45	0.69	0.09	0.60	1.17
12	0.76	1.19	0.85	missing	0.68	0.16	0.73	1.25
24	0.57	0.98	0.65	0.84	0.19	0.24	0.58	0.78
32	0.41	0.43	0.28	0.74	0.39	0.17	0.62	0.38
48	0.28	0.37	0.21	0.49	0.36	missing	0.28	0.14
72	0.11	0.06	0.10	0.21	0.12	0.10	0.13	0.07
96	0.16	0.04	0.09	0.08	missing	0.16	0.08	0.11
120	0.08	0.09	0.05	0.07	0.09	0.17	0.07	0.11
144	missing	UD	missing	UD	0.06	0.03	missing	missing

UD = undetectable (<0.05 μg/ml)

Table A-7.
The concentrations (μg/ml) of flunixin meglumine (FM) in plasma following intravenous administration of FM at 1.1 mg/kg in sheep.

	Animal numbers							
Time (h)	143	144	145	146	147	148	149	150
0.08	16.51	26.89	22.18	14.12	22.95	24.19	11.90	25.10
0.25	11.56	18.33	16.61	10.66	12.71	16.78	7.93	19.60
0.50	8.74	13.19	12.08	5.13	10.55	11.79	6.63	13.89
0.75	6.15	10.11	9.37	5.18	8.06	9.81	5.42	11.72
1.00	4.79	9.40	7.82	4.09	6.55	8.21	3.89	9.58
2.00	2.74	5.60	4.40	2.14	3.42	5.25	2.05	5.33
4.00	1.40	3.13	2.27	2.20	1.28	2.98	2.12	3.11
6.00	0.80	1.89	1.14	0.84	0.66	1.48	0.79	1.15
8.00	0.50	1.12	1.09	0.48	0.33	0.75	0.64	0.72
12.00	0.13	0.26	0.26	0.19	0.07	0.38	0.21	0.41
24.00	UD	0.06	UD	UD	UD	0.06	UD	0.06
32.00	UD	UD	UD	UD	UD	0.05	UD	UD
48.00	UD	UD	UD	UD	UD	UD	UD	UD

UD = undetectable (<0.05 μg/ml)

Table A-8.
The concentrations (μg/ml) of flunixin meglumine (FM) in exudate following intravenous administration of FM at 1.1 mg/kg in sheep.

	Animal numbers							
Time(h)	143	144	145	146	147	148	149	150
2	0.37	0.80	0.64	0.49	0.85	0.40	0.20	1.86
4	0.97	2.66	1.77	1.03	2.04	1.12	0.83	2.70
8	0.89	2.55	1.47	1.52	1.45	1.57	1.32	1.92
12	0.36	1.35	1.15	1.01	0.48	1.47	0.89	0.82
24	0.25	0.80	0.59	0.51	0.20	0.69	0.44	0.23
32	0.20	0.26	0.17	0.41	UD	0.30	0.32	UD
48	UD	UD	UD	UD	UD	UD	UD	UD

UD = undetectable (<0.05 μg/ml)

Table A-9.
The concentrations (μg/ml) of flunixin meglumine (FM) in transudate following intravenous administration of FM at 1.1 mg/kg in sheep.

	Animal numbers							
Time(h)	143	144	145	146	147	148	149	150
2	0.32	0.33	0.56	0.63	0.45	0.55	0.17	0.38
4	0.91	2.62	1.33	1.07	1.44	1.78	0.97	0.07
8	0.98	3.55	1.43	1.11	1.11	1.01	1.00	1.27
12	0.50	1.83	0.59	1.05	0.89	1.89	0.90	0.87
24	0.24	1.50	0.81	0.51	0.50	0.67	0.71	0.40
32	0.14	0.33	0.16	UD	0.25	0.41	0.31	0.19
48	0.09	0.23	UD	UD	0.19	0.25	0.19	UD

UD = undetectable (<0.05 μg/ml)

Table A-10
The changes in skin temperature (°C) over the cages following intravenous administration of placebo in sheep (the values are expressed as the difference between inflamed and non-inflamed cages).

	Animal numbers							
Time	143	144	145	146	147	148	149	150
-20 min	-0.2	-1.8	-1.7	-1.7	1.8	0.7	0.2	0.2
1h	0.0	-3.6	0.6	0.3	1.9	0.2	-1.8	-2.0
2h	0.4	1.2	1.4	1.3	-1.1	3.4	1.4	1.6
4h	2.0	0.9	2.9	0.7	0.1	2.3	1.3	2.0
6h	0.0	0.6	2.5	-0.4	0.2	2.3	0.7	1.4
8h	1.1	1.5	-1.0	-2.7	0.4	2.0	1.1	0.4
12h	0.0	2.2	1.9	-0.5	-0.4	2.7	2.1	0.5
24h	0.7	0.4	1.6	-0.9	-1.0	1.2	-0.6	1.8
32h	0.9	0.5	1.5	-2.4	-0.3	1.3	-0.3	1.2
48h	-0.1	1.7	0.5	-0.3	-0.1	2.9	0.4	2.0
72h	2.2	0.7	0.3	-0.8	-0.8	-0.1	-0.7	1.3
96h	0.9	0.9	1.7	0.3	0.4	1.3	-0.6	0.3
120h	-0.1	0.2	0.1	-0.5	-0.8	-1.0	0.2	1.9
144h	-0.1	0.2	1.4	0.8	-0.1	-1.0	0.2	2.6

Table A-11
The changes in skin temperature (°C) over the cages following intravenous administration of phenylbutazone at 4.4 mg/kg in sheep (the values are expressed as the difference between inflamed and non-inflamed cages).

	Animal numbers							
Time	143	144	145	146	147	148	149	150
-20 min	-1.1	0.8	2.8	-1.5	-4.5	-1.1	2.5	0.4
1h	-0.3	1.8	0.7	0.9	1.6	3.4	1.7	0.4
2h	-0.3	0.2	-0.9	-1.3	1.3	-2.2	0.8	-0.1
4h	-0.2	2.6	0.0	-1.1	2.2	-0.2	1.5	1.3
6h	2.9	0.2	0.7	-0.9	-0.8	0.0	2.9	0.7
8h	0.2	0.9	-0.7	-0.4	-1.3	1.0	0.9	0.8
12h	-1.5	0.3	-0.4	1.0	0.0	0.2	-1.1	1.4
24h	-0.6	0.5	1.2	-0.3	4.4	-0.8	0.8	0.0
32h	-1.2	0.9	2.8	0.7	-3.2	-1.2	2.0	1.8
48h	-0.8	0.2	2.9	-1.8	0.8	1.8	0.6	0.4
72h	-0.6	-0.3	0.3	-2.2	1.4	-2.3	1.3	0.0
96h	1.0	-0.8	2.9	0.5	0.9	-1.8	0.9	0.0
120h	1.2	0.5	1.1	-0.1	-1.8	-0.6	1.1	1.8
144h	2.3	0.5	0.7	-0.1	1.2	-0.9	2.3	-0.3

Table A-12
The changes in skin temperature (°C) over the cages following intravenous administration of flunixin meglumine at 1.1 mg/kg in sheep (the values are expressed as the difference between inflamed and non-inflamed cages).

	Animal numbers							
Time	143	144	145	146	147	148	149	150
-20 min	-0.2	-1.2	1.5	-2.0	-0.4	1.7	-0.2	0.5
1h	2.3	-1.3	0.1	0.7	-0.4	1.1	-2.6	-0.2
2h	-1.8	0.2	-0.5	-2.1	1.8	3.4	-1.3	-1.3
4h	-0.2	-0.1	-0.2	-1.1	-2.0	-2.5	0.8	-2.4
6h	-0.2	0.5	0.4	-1.6	-0.2	-2.0	0.9	-2.4
8h	-0.6	-1.9	0.9	-0.2	-0.2	0.9	0.9	0.1
12h	1.6	0.4	1.2	0.4	0.0	1.4	1.2	-1.9
24h	2.2	-0.4	1.9	0.1	1.1	1.4	1.3	0.2
32h	-1.6	-2.0	0.8	1.5	0.1	-1.1	-1.0	0.4
48h	3.7	0.7	0.0	0.7	-0.1	-0.6	1.3	0.6
72h	0.5	0.0	1.3	1.0	-1.2	3.4	1.5	-0.1
96h	1.9	-1.3	-0.2	-0.4	-0.4	2.4	0.8	0.1
120h	-0.7	-1.3	0.9	-1.2	-0.5	0.4	-1.1	0.5
144h	-2.0	-1.4	0.9	-3.0	-0.6	2.5	-0.5	2.7

Table A-13.
The concentrations (ng/ml) of serum thromboxane B_2 following intravenous administration of placebo in sheep.

	Animal number							
Time	143	144	145	146	147	148	149	150
-20 min	20.36	20.09	23.78	20.02	10.91	19.65	30.55	22.07
1 h	8.87	19.08	19.06	9.21	7.76	16.29	8.42	19.03
2 h	17.04	20.70	16.36	8.03	9.75	18.89	31.86	19.03
4 h	16.35	17.37	22.97	13.36	9.74	18.11	29.19	16.83
6 h	14.12	13.92	16.78	11.79	6.43	15.99	29.33	17.84
8 h	16.42	18.56	15.89	11.03	8.73	16.48	29.50	17.13
12 h	11.43	13.74	12.57	8.41	7.60	14.82	28.95	13.43
24 h	12.70	17.73	16.34	13.44	7.84	18.09	30.00	14.57
32 h	14.95	17.04	24.21	14.30	15.91	19.57	28.61	21.89
48 h	17.03	21.06	25.37	17.11	10.62	20.69	29.58	22.59
72 h	20.79	20.24	25.79	18.30	16.48	21.05	31.93	27.42
96 h	21.56	22.14	31.71	21.56	20.93	22.86	31.00	24.14
120 h	24.87	22.12	21.83	21.87	20.92	22.67	32.34	26.17
144 h	23.99	24.56	26.38	22.71	23.75	24.35	32.03	22.65

Table A-14.
The concentrations (ng/ml) of serum thromboxane B_2 following intravenous administration of phenylbutazone at 4.4 mg/kg in sheep.

	Animal numbers							
Time	143	144	145	146	147	148	149	150
-20 min	11.48	27.33	31.51	18.28	20.79	14.89	44.42	27.28
1 h	UD	7.25	1.40	5.02	3.49	1.43	11.04	28.70
2 h	UD	7.54	1.37	4.01	2.37	UD	17.07	12.54
4 h	2.96	12.09	2.65	4.84	5.72	2.21	30.09	11.95
6 h	2.96	7.12	1.37	2.78	3.47	UD	21.38	13.21
8 h	2.24	9.57	2.50	4.65	4.68	1.66	14.78	13.27
12 h	1.49	8.12	3.44	4.31	10.52	1.83	13.65	12.72
24 h	7.17	14.75	11.27	8.96	17.85	4.07	41.70	21.45
32 h	3.24	15.57	10.33	11.82	12.91	6.04	21.68	20.25
48 h	9.22	28.68	20.13	18.83	20.41	9.83	58.71	26.28
72 h	16.10	23.94	28.47	20.53	22.09	17.24	51.74	27.55
96 h	18.18	26.42	26.21	19.84	37.02	16.79	5.68	29.34
120 h	17.88	26.18	33.62	24.43	36.97	17.19	52.22	28.93
144 h	17.27	24.39	28.38	25.70	26.72	15.94	51.57	28.84

UD = undetectable (<0.8 ng/ml)

Table A-15.
The concentrations (ng/ml) of serum thromboxane B_2 following intravenous administration of flunixin meglumine at 1.1 mg/kg in sheep.

Time	Animal number							
	143	144	145	146	147	148	149	150
-20 mins	11.12	25.27	22.45	22.01	21.55	22.81	35.47	29.81
1 h	UD	UD	UD	UD	UD	UD	UD	UD
2 h	UD	UD	UD	UD	UD	UD	UD	UD
4 h	UD	UD	UD	UD	UD	UD	UD	UD
6 h	UD	UD	UD	UD	UD	UD	UD	UD
8 h	UD	UD	UD	UD	UD	UD	UD	UD
12 h	UD	UD	UD	UD	UD	UD	0.67	UD
24 h	1.92	4.73	13.88	3.18	10.74	4.79	22.32	8.32
32 h	6.44	15.04	17.71	10.27	15.14	10.68	27.19	19.27
48 h	8.86	27.28	27.07	18.15	19.17	18.30	33.96	27.63
72 h	6.75	30.12	31.12	17.28	17.58	23.56	38.32	29.39
96 h	10.59	29.84	28.51	23.58	17.52	24.46	35.00	32.60
120 h	9.32	28.25	27.17	21.24	18.88	20.35	33.85	26.99
144 h	17.80	28.35	26.26	19.54	17.69	24.57	33.21	25.09

UD = undetectable (<0.8 ng/ml)

Table A-16.
The concentrations (ng/ml) of exudate prostaglandin E_2 following intravenous administration of placebo in sheep.

Time	Animal numbers							
	143	144	145	146	147	148	149	150
-20 min	UD	UD	UD	UD	UD	UD	UD	UD
2 h	0.32	UD	0.28	UD	1.81	UD	19.55	11.00
4 h	1.41	16.05	2.29	3.88	27.64	2.08	41.10	25.52
8 h	38.79	27.14	28.31		45.88	29.56	42.44	27.62
12 h	55.40	27.68	34.72	9.25	46.60	37.69	43.09	30.79
24 h	33.71	32.53	32.76	17.06	44.16	22.80	28.71	20.85
32 h	45.47	12.08	11.71	9.29	26.53	7.66	30.80	16.01
48 h	6.89	3.83	0.88	2.91	16.53	0.68	20.50	17.09
72 h	1.28	0.92	0.49	1.41	3.98	0.00	31.08	21.69
96 h	1.15	0.00	0.513	5.42	0.00	0.00	25.35	29.31
120 h	1.95	0.00	0.75	5.91	1.02	0.52	31.80	29.50
144 h	8.33	2.12				0.47		

UD = undetectable (<0.8 ng/ml)

Table A-17.
The concentrations (ng/ml) of exudate prostaglandin E_2 following intravenous administration of phenylbutazone at 4.4 mg/kg in sheep.

Time	Animal number							
	143	144	145	146	147	148	149	150
-20 min	UD	UD	1.80	UD	UD	UD	UD	UD
2 h	0.33	UD	1.11	UD	UD	UD	UD	UD
4 h	1.04	1.59	22.23	16.52	0.49	14.69	0.05	0.73
8 h	39.66	41.17	37.53	40.26	8.20	39.01	7.45	17.90
12 h	43.63	17.84	7.67	37.88	8.32	33.26	7.65	20.16
24 h	41.33	2.69	35.11	37.93	4.91	31.04	6.63	32.73
32 h	37.21	1.72	21.12	7.99	1.34	34.94	2.31	7.62
48 h	6.58	0.00	21.03	0.99	0.24	29.26	0.28	1.07
72 h	0.96	0.00	9.56	0.00	0.40	1.15	0.26	0.43
96 h	0.59	0.00	7.11	0.00	4.24	0.27	0.23	0.59
120 h	1.15	0.00	7.61	0.00	0.06	0.66	2.31	0.73
144 h		1.15	0.00			1.78		1.02

UD = undetectable (<0.8 ng/ml)

Table A-18.
The concentrations (ng/ml) of exudate prostaglandin E_2 following intravenous administration of flunixin meglumine at 1.1 mg/kg in sheep.

Time	Animal numbers							
	143	144	145	146	147	148	149	150
-20 min	UD	UD	UD	UD	UD	UD	UD	UD
2 h	UD	UD	UD	UD	UD	UD	UD	UD
4 h	UD	UD	UD	UD	UD	UD	UD	UD
8 h	UD	UD	UD	1.47	UD	UD	UD	UD
12 h	1.00	UD	UD	11.79	UD	UD	0.42	0.28
24 h	0.77	UD	UD	21.01	UD	UD	3.45	0.23
32 h	3.96	UD	UD	19.68	1.42	UD	12.20	0.46
48 h	2.33	UD	UD	2.02	0.51	UD	11.19	0.75
72 h	1.61	UD	UD	0.92	0.43	UD	3.29	3.10
96 h	6.37	UD	UD	1.94	0.58	UD	4.33	0.31
120 h	4.31	UD	UD	2.25	0.75	UD	6.88	4.06
144 h	9.70	UD	UD	UD	0.74	UD	UD	UD

UD = undetectable (<0.8 ng/ml)

Table A-19.
The concentrations (ng/ml) of exudate leukotriene B_4 following intravenous administration of placebo in sheep.

	Animal numbers							
Time (h)	143	144	145	146	147	148	149	150
2	0.45	0.46	2.57	1.80	0.54	0.20	0.50	1.61
4	0.60	0.85	1.91	2.99	1.32	0.23	0.97	1.67
8	0.71	0.99	2.26	3.44	0.53	0.55	0.92	1.21
12	0.88	2.05	1.90	3.25	1.04	0.66	1.04	2.20
24	0.45	1.09	2.08	2.22	0.51	0.39	0.74	1.12
32	0.90	0.73	2.01	2.43	1.01	0.29	0.55	1.51
48	0.80	0.83	2.24	1.77	0.57	0.28	0.94	1.58
72	0.97	0.76	2.61	3.46	0.47	0.26	1.02	2.17
96	0.95	0.77	1.85	3.85	0.74	0.33	1.06	2.25
120	0.71	0.51	1.57	3.08	1.05	0.35	0.84	1.77
144	0.70	1.11				0.46		

Table A-20.
The concentrations (ng/ml) of exudate leukotriene B_4 following intravenous administration of phenylbutazone at 4.4 mg/kg in sheep.

	Animal numbers							
Time (h)	143	144	145	146	147	148	149	150
2	1.67	0.35	0.60	0.32	2.21	1.08	2.28	0.27
4	2.12	0.37	0.85	0.52	2.50	0.88	1.82	0.54
8	1.38	0.82	0.96	0.84	2.64	0.76	1.83	0.55
12	1.76	0.72	1.66	1.05	2.63	0.90	2.30	0.43
24	1.93	0.40	0.79	0.53	2.43	0.74	2.48	0.43
32	1.58	0.44	0.67	0.54	2.73	1.79	2.81	0.62
48	1.94	0.33	0.82	0.53	2.53	1.64	3.20	0.69
72	2.28	0.59	0.72	0.55	4.46	1.60	3.08	0.40
96	1.92	0.44	1.06	0.50	3.40	1.65	3.22	0.68
120	1.92	0.46	1.07	0.48	2.44	1.49	3.33	0.53
144		0.79	0.94			2.06		1.14

Table A-21.
The concentrations (ng/ml) of exudate leukotriene B_4 following intravenous administration of flunixin meglumine at 1.1 mg/kg in sheep.

	Animal numbers							
Time (h)	143	144	145	146	147	148	149	150
2	0.79	2.06	0.39	0.70	0.59	1.90	0.87	1.47
4	1.07	2.42	0.55	0.98	0.66	2.16	1.07	2.17
8	1.31	2.47	0.62	1.28	0.56	2.08	1.82	2.67
12	1.31	1.82	0.58	1.44	0.57	2.52	1.84	2.30
24	0.68	2.70	0.59	1.17	0.32	2.26	1.81	2.33
32	0.73	2.24	0.68	0.84	0.59	2.23	1.77	1.91
48	0.65	2.14	0.46	0.65	0.34	3.20	1.62	2.53
72	0.81	2.26	0.49	0.79	0.37	1.70	1.58	2.06
96	1.25	2.56	0.77	0.55	0.53	2.28	1.66	2.45
120	1.03		0.69	1.14	1.16	3.08	1.54	2.21
144	0.80		0.65		0.51			

Table A-22.
Leukocyte numbers (× 10^9 cells/L) in exudate following intravenous administration of placebo in sheep.

	Animal numbers							
Time(h)	143	144	145	146	147	148	149	150
4	4.9	3.3	6.3	5.0	7.1	6.3	10.6	29.9
8	12.3	7.5	8.9	10.2	40.5	29.1	15.6	22.5
12	23.2	19.9	25.1	14.6	23.8	64.8	26.1	15.7
24	37.6	26.1	18.0	68.7	24.5	89.2	23.2	48.3
48	5.6	14.5	3.8	12.6	18.9	18.9		26.1

Table A-23.
Leukocyte numbers (× 10^9 cells/L) in exudate following intravenous administration of phenylbutazone at 4.4 mg/kg in sheep.

	Animal numbers							
Time(h)	143	144	145	146	147	148	149	150
4	4.6	10.4	4.5	6.2	8.3	2.5	7.8	7.7
8	10.8	4.8	13.0	16.7	10.2	12.1	17.1	12.8
12	17.8	16.0	14.1	32.2	7.7	17.9	37.7	18.8
24	26.0	47.7	40.5	17.5	14.5	42.8	5.4	18.4
48	7.2	12.8	17.4	7.7	3.1	5.7	5.6	7.4

Table A-24.
Leukocyte numbers (× 10^9 cells/L) in exudate following intravenous administration of flunixin meglumine at 1.1 mg/kg in sheep.

	Animal numbers							
Time(h)	143	144	145	146	147	148	149	150
4	2.4	5.6	8.0	4.6	7.4	9.7	1.9	6.1
8	10.5	14.5	12.3	18.4	7.0	25.1	5.6	9.2
12	15.7	10.5	28.4	23.3	17.8	17.1	6.5	6.5
24	34.3	31.0	42.7	64.5	15.4	74.4	22.1	15.5
48	9.1	6.2	10.8	19.6	4.7	8.1	7.6	3.7

Table A-25.
Leukocyte numbers (× 10^9 cells/L) in venous blood following intravenous administration of placebo in sheep.

	Animal numbers							
Time	143	144	145	146	147	148	149	150
-20 min	6.0	5.7	7.2	5.4	8.6	6.1	6.9	7.3
4 h	5.6	5.2	7.0	5.8	7.2	5.6	6.8	9.1
24 h	7.2	6.9	8.6	6.8	10.0	9.1	6.9	10.9
48 h	6.4	5.8	7.5	5.7	8.7	6.3	6.4	10.0

Table A-26.
Leukocyte numbers (× 10^9 cells/L) in venous blood following intravenous administration of phenylbutazone at 4.4 mg/kg in sheep.

	Animal numbers							
Time	143	144	145	146	147	148	149	150
-20 min	6.4	5.4	5.6	4.4	8.4	CLOT	7.1	9.5
4 h	6.2	8.2	6.1	4.6	9.2	6.4	6.4	6.8
24 h	7.2	9.1	5.8	6.2	9.3	8.3	6.8	9.3
48 h	6.5	6.3	5.9	5.5	9.1	7.3	5.4	8.7

Table A-27.
Leukocyte numbers (× 10^9 cells/L) in venous blood following intravenous administration of flunixin meglumine at 1.1 mg/kg in sheep.

	Animal numbers							
Time	143	144	145	146	147	148	149	150
-20 min	4.5	5.9	6.6	5.5	7.8	6.4	6.1	9.6
4 h	4.5	5.0	8.9	5.1	7.0	7.2	5.7	9.3
24 h	5.9	5.8	8.3	6.4	9.2	8.0	3.6	10.7
48 h	5.2	9.8	7.1	5.2	7.5	7.1	10.0	9.8

Table A-28.
Platelet numbers (× 10^9 cells/L) in venous blood following intravenous administration of placebo in sheep.

Time	Animal numbers							
	143	144	145	146	147	148	149	150
-20 min	199.0	216.0	270.0	254.0	234.0	245.0	116.0	73.0
4 h	211.0	239.0	278.0	227.0	234.0	201.0	139.0	47.0
24 h	217.0	213.0	301.0	224.0	214.0	240.0	196.0	31.0
48 h	207.0	252.0	309.0	226.0	278.0	231.0	135.0	45.0

Table A-29.
Platelet numbers (× 10^9 cells/L) in venous blood following intravenous administration of phenylbutazone at 4.4 mg/kg in sheep.

Time	Animal numbers							
	143	144	145	146	147	148	149	150
-20 min	229.0	241.0	256.0	93.0	183.0		265.0	78.0
4 h	217.0	159.0	254.0	210.0	259.0	184.0	202.0	95.0
24 h	225.0	222.0	263.0	217.0	265.0	186.0	72.0	75.0
48 h	227.0	219.0	274.0	218.0	242.0	241.0	70.0	81.0

Table A-30.
Platelet numbers (× 10^9 cells/L) in venous blood following intravenous administration of flunixin meglumine at 1.1 mg/kg in sheep.

Time	Animal numbers							
	143	144	145	146	147	148	149	150
-20 min	188.0	254.0	246.0	149.0	260.0	36.0	220.0	73.0
4	239.0		252.0	169.0	247.0	154.0	212.0	
24	198.0	232.0	124.0	164.0	233.0	90.0	36.0	67.0
48	197.0	253.0	248.0	167.0	241.0	158.0	159.0	70.0

Appendix B

Pharmacodynamics of carprofen and its enantiomers in sheep
Expermental code: QM/ZC/03/95

Table B-1.
Skin temperature (°C) over the cages following intravenous administration of placebo in sheep (Values are expressed as the difference between inflamed and non-inflamed tissue-cages).

	Animal numbers							
Time (h)	143	144	145	146	147	148	149	150
-0.33		-0.8	0.0	0.3	0.2	0.8		0.0
1	-0.6	-0.8	-1.2	1.1	1.0	0.2	1.7	0.9
2	-0.7	0.8	0.6	0.5	1.5	-0.1	0.4	-0.7
4	1.4	-2.4	-0.4	0.7	0.5	0.1	1.3	0.8
6	1.0	0.1	1.6	0.1	0.9	0.6	1.1	0.4
8	1.0	0.7	1.7	0.3	0.2	0.5	1.1	0.4
12	1.7	0.2	1.1	1.4	0.1	0.4	1.3	4.4
24	0.1	-0.3	-0.6	1.0	0.1	0.7	0.8	1.6
32	0.9	0.3	-0.3	-0.1	-0.5	-0.2	0.5	1.7
48	1.1	-0.7	-0.2	-0.1	-0.8	0.7	0.2	1.1
72	1.3	0.7	0.4	-1.0	0.5	0.7	-1.1	0.4
96	1.5	-1.9	-0.7	-0.2	1.1	-0.8	0.5	0.9
120	1.0	-0.2	-0.8	-0.2	1.1	-0.5	0.6	1.3
144	-0.8	-0.7	0.8	1.4	1.2	0.0	0.8	1.2

Table B-2.
Skin temperature (°C) over the cages following intravenous administration of racemic carprofen at 4.0 mg/kg in sheep (Values are expressed as the difference between inflamed and non-inflamed tissue-cages).

	Animal numbers							
Time(h)	143	144	145	146	147	148	149	150
-0.33	-0.6	-0.3	-0.2		0.6	0.4	0.3	
1	-1.5	-0.3		-0.3	-0.1	1.0	-0.6	
2	-1.0	0.0	2.3	-0.7	-0.4	-0.1	2.0	1.2
4	0.1	0.0	1.3	-0.2	1.0	1.1	0.9	0.2
6	0.4	0.1	2.9	0.2	0.7	0.6	-0.6	0.6
8	-0.1	0.0	2.3	-0.3	0.5	1.3	0.2	0.5
12	1.0	-1.4	1.4	0.7	0.2	2.1	-0.7	2.3
24	1.4	-0.5	0.8	-1.1	0.4	1.9	-1.7	-0.2
32	0.0	-1.3	0.8	2.5	0.9	0.9	0.6	-0.1
48	1.3	0.6	0.7	0.4	1.6	1.8	0.5	-0.5
72	-1.7	0.0	1.8	0.9	-0.9	0.8	-0.6	-0.8
96	0.0	-0.1	0.5	1.8	0.0	1.0	-0.5	-0.1
120		0.0	1.5	0.3	-1.1		-0.1	0.3
144	1.8	-1.1	0.6	-0.1	0.3	-0.2	0.8	-0.5

Table B-3.
Skin temperature (°C) over the cages following intravenous administration of R(-) carprofen at 2.0 mg/kg in sheep (Values are expressed as the difference between inflamed and non-inflamed tissue-cages).

	Animal numbers							
Time(h)	143	144	145	146	147	148	149	150
-0.33		0.5	-0.7	0.5		-0.9	-1	0.9
1	-2.1	2.2	0.2		-0.7	0	0.1	0.9
2	1	1	0.2	0.8	1.4	0.3	-0.3	0.8
4	-0.8	1.2	0.3	0.7	0.1	0.3	-1.2	3.1
6	-0.4	0.7	0.5	1.7	0.5	0.6	0.8	1.2
8	1.8	0.7	-0.1	0.6	0.6	0.2	0.8	0
12	0.6	0.8	0.3	1.9	0.5	0.8	2	0
24	1.4	-0.5	-0.4	0.4	-0.5	-1.5	1	0.2
32	0.8	-0.1	-1.2	0.5	0.5	0.2	1.5	-0.2
48	1.8	-0.4	0.5	0.7	1.1	-0.5	1.2	-0.8
72	0.2	-0.6	-0.7	-0.2	0.6	-0.7	1.2	-0.3
96	0.1	-0.2	0.4	0.2	1.8	-0.4	1.2	-0.1
120	0.8		0.1	0.8	1.1	-0.6		0.4
144	1.4	-2.1	0.8	2.2	0.6	-0.4	2	1.1

Table B-4.
Skin temperature (°C) over the cages following intravenous administration of S(+) carprofen at 2.0 mg/kg in sheep (Values are expressed as the difference between inflamed and non-inflamed tissue-cages).

	Animal numbers							
Time(h)	143	144	145	146	147	148	149	150
-0.33	0.6	-0.7	1.3	0.9	-0.7	0.1		1.2
1		0.1	-0.8	0.2		-0.5	0.1	0.2
2	1.6	0.6	1.5	-0.2	1.2	0.5	0.6	-0.4
4	0.7	-0.2	1.7	0.4	0.7	2.0	0.4	0.6
6	0.2	0.0	0.7	-0.6	1.2	1.3	0.4	1.5
8	1.2	0.1	0.1	0.3	-1.5	-0.1	1.3	-0.4
12	0.6	0.8	2.4	0.9	1.0	-0.1	1.2	0.0
24	0.0	0.2	1.4	-1.8	0.6	0.3	0.7	0.8
32	0.5	-0.2	1.3	1.1	-0.8	2.0	0.7	-0.1
48	1.0	0.2	-1.2	0.4	0.4	3.2	0.0	0.4
72	0.5	-0.4	0.7	-1.4	0.1	0.5	1.0	0.1
96	0.6	0.0	0.5	1.2	0.0	0.6	0.7	0.3
120	-0.7	0.0		1.1	-0.9	-0.3	0.3	
144	0.0	0.6	0.7	1.5	0.6	0.9	0.8	1.8

Table B-5.
Skin temperature (°C) over the cages following intravenous administration of N^G-nitro-L-arginine methyl ester at 25 mg/kg in sheep (Values are expressed as the difference between inflamed and non-inflamed tissue-cages).

	Animal numbers							
Time(h)	143	144	145	146	147	148	149	150
-0.33	-0.1	0.8	1.0	1.4	-0.4	-0.7	1.1	0.4
1	0.1	0.5	-1.4	0.2	-1.0		0.3	0.0
2	-0.6	0.4	0.1	0.1	0.1	1.5	0.5	-1.6
4	1.0	-0.1	-0.3	-0.9	-0.6	0.7	-0.1	1.4
6	0.3	0.9	0.1	0.7	0.4	0.3	-0.2	-0.2
8	0.3	4.1	-0.4	-0.5	-0.2	0.4	-1.2	0.5
12	0.3	3.0	-0.3	-0.6	0.1	0.6	-0.7	0.8
24	-1.0	0.2	-0.4	1.4	-0.3	-0.1	1.0	-1.4
32	-0.6	1.0	-1.1	0.1	0.0	0.5	0.6	0.0
48	0.2	1.8	-0.2	1.0	-0.5	0.9	-0.8	-0.1
72	1.1	-0.6	-0.4	0.1	-0.9	0.4	0.5	0.1
96	0.1	2.4	-0.7	1.4	-0.1	1.1	0.7	-0.6
120		3.3			-1.0	0.7	-0.1	
144	0.2	1.9	-1.1	1.0	0.2	0.7	0.1	1.6

Table B-6.
Concentrations of serum thromboxane B_2 (ng/ml) following intravenous administration of placebo in sheep.

	Animal numbers							
Time (h)	143	144	145	146	147	148	149	150
-0.33	20.24	32.37	26.67	27.89	24.22	20.23	32.70	32.70
1	15.35	33.04	27.83	19.98	19.17	20.53	32.56	31.46
2	16.46	35.51	32.20	20.15	19.13	22.42	33.95	31.00
4	14.03	32.00	29.24	23.19	20.28	27.59	29.68	31.98
6	14.96	33.14	29.17	23.14	20.14	24.24	31.16	29.83
8	16.27	31.91	28.34	21.91	35.72	25.48	34.01	29.45
12	15.08	30.42	29.00	22.67	22.06	22.00	33.38	30.30
24	15.38	32.27	31.33	22.46	25.94	25.09	35.26	25.92
32	14.65	32.25	31.88	20.44	26.19	19.77	35.23	30.48
48	20.62	33.24	17.72	15.28	21.74	25.22	34.84	30.71
72	24.11	29.71	31.14	19.56	23.83	26.07	34.42	27.90
96	21.99	45.84	23.28	34.10	34.89	16.71	61.02	50.99
120	16.26	51.47	25.62	31.98	22.70	23.56	57.67	49.18
144	24.45	40.63	55.46	28.82	28.25	35.99	60.17	50.62

Table B-7.
Concentrations of serum thromboxane B_2 (ng/ml) following intravenous administration of racemic carprofen at 4.0 mg/kg in sheep.

	Animal numbers							
Time (h)	143	144	145	146	147	148	149	150
-0.33	27.43	29.07	30.96	32.46	17.05	32.16	31.09	35.35
1	2.97	3.95	6.06	7.33	1.61	8.50	3.81	11.47
2	5.24	4.23	9.48	8.92	2.49	10.65	4.15	11.41
4	5.86	9.78	1.26	10.78	3.93	8.79	7.19	15.06
6		7.86		21.82	4.59		8.48	
8	12.63	15.99	16.47	16.31	5.29	19.52	12.39	17.38
12	6.45	5.82	14.86	19.84	9.81	10.83	15.66	17.28
24	24.62	17.64	28.42	25.47	11.79	21.79	23.54	18.34
32	16.66	26.68	30.45	42.58	12.31	19.82	16.29	20.29
48	30.09	29.31	27.95	21.63	18.51	27.62	31.32	29.76
72	30.46	28.82	25.51	31.64	18.33	33.99	34.65	31.74
96	41.07	40.42	44.57	44.46	23.06	30.93	36.11	32.38
120	48.96	38.73	36.59		3.48	31.69	32.74	31.86
144	39.20	37.83			15.06	33.12	35.02	

Table B-8.
Concentrations of serum thromboxane B_2 (ng/ml) following intravenous administration of R(-) carprofen at 2.0 mg/kg in sheep.

	Animal numbers							
Time (h)	143	144	145	146	147	148	149	150
-0.33	22.69	38.67	27.74	38.27	18.99	27.40	37.68	26.58
1	28.93	35.89	16.31	31.65	17.57	25.61	33.89	27.75
2	29.43	33.38	22.24	32.49	21.86	28.99	35.99	25.34
4	28.65	38.11	9.57	31.94	22.35	30.69	37.16	25.51
6	27.72		19.60		17.75	25.00	30.07	24.89
8	27.79	36.39	18.67	34.94	17.66	25.14		23.50
12	31.52	39.13	7.61	27.66	21.38	27.99	37.07	22.68
24	30.09	39.07	23.93	29.59	22.59	23.11	33.67	28.74
32	26.67	39.43	18.86	33.08	27.51	25.83	35.52	26.62
48	25.99	41.24	26.98	32.31	20.07	23.21	38.05	25.09
72	36.04	38.94	28.91	33.08	24.61	26.78		28.13
96	27.78	39.45	16.21	33.42	18.57	24.09	39.23	29.31
120		40.19	5.12	32.40		20.91		28.43
144		31.98	32.18			24.32	38.62	26.52

Table B-9.
Concentrations of serum thromboxane B_2 (ng/ml) following intravenous administration of S(+) carprofen at 2.0 mg/kg in sheep.

	Animal numbers							
Time (h)	143	144	145	146	147	148	149	150
-0.33	28.05	32.63	29.28	25.90	30.16	29.07	36.86	32.96
1	7.27	7.08	3.66	1.13	3.77	27.07	17.19	12.64
2	9.36	7.64	7.96	1.19	6.41	20.74	18.88	16.81
4	10.96		9.57	2.63	3.66	20.33	23.52	21.79
6		13.33		2.81		11.83	21.26	21.79
8	14.84	7.19	15.69	3.84	10.28	19.86	23.79	
12	13.02	13.89	12.76	3.89	11.29	13.93	22.85	19.51
24	23.20	14.76	25.76	10.44	10.48	26.41	27.04	29.49
32	22.74	14.76	27.98	11.22	7.99		28.18	29.55
48	27.87	24.47	31.57	17.16	27.09	38.08	32.03	32.52
72	32.05	22.67	33.64	19.41	27.73	33.70	34.69	33.91
96	31.86		34.08	15.71	27.36	29.92		35.50
120	28.17	30.58	34.74	15.38	33.73		20.31	33.67
144	30.08	15.52	33.12	17.40			36.01	35.24

Table B-10.
Concentrations of serum thromboxane B_2 (ng/ml) following intravenous administration of N^G-nitro-L-arginine methyl ester at 25 mg/kg in sheep.

	Animal numbers							
Time (h)	143	144	145	146	147	148	149	150
-0.33	20.07	32.14	26.11	29.96	31.63	32.18	34.98	32.68
1	12.61	27.63	15.12	25.53	12.66	25.25	35.53	25.30
2	13.11	27.57	16.17	25.56	19.77	27.48	33.81	26.43
4	12.44	28.51	15.04	27.59	13.59	32.23	34.56	26.55
6	18.33	26.52	17.95	27.57	15.65	26.62	33.55	24.02
8	11.45	28.55	21.82	25.82	12.41	23.25	34.18	26.16
12	14.61	29.75	13.97	27.33	14.67	31.68	32.79	26.94
24	21.52	31.07	15.86	28.57	25.65	32.54	33.85	23.78
32	18.30	32.63	15.99	29.40	19.32	33.25	34.12	26.23
48	18.66	28.15	12.02	28.86	18.03	27.62	28.38	25.56
72	18.70	31.87	23.39	30.02	20.03	27.50	35.51	27.20

Table B-11.
Concentrations of exudate prostaglandin E_2 (ng/ml) following intravenous administration of placebo in sheep.

	Animal numbers							
Time (h)	143	144	145	146	147	148	149	150
2	UD	2.17	4.34	UD	UD	UD	55.25	UD
4	21.59	11.35	90.61	12.13	47.98	14.85	89.86	UD
8	93.24	68.26	111.43	94.86	101.35	96.56	57.72	14.81
12	115.32	78.97	108.90	97.74	97.61	112.64	61.34	17.35
24	110.86	68.39	89.06	102.19	51.85		9.78	5.05
32	76.11	38.45	40.02	75.24	12.75	47.22	5.59	10.80
48		1.93	9.38	14.52		2.31	UD	19.79
72	4.19	1.90	UD		UD	15.30	UD	24.10
96	4.96		2.29				UD	2.02
120					UD	UD		55.18
144	2.09	UD	UD	1.46	UD	UD	UD	32.74

UD = undetectable (<0.8 ng/ml)

Table B-12.
Concentrations of exudate prostaglandin E_2 (ng/ml) following intravenous administration of racemic carprofen at 4.0 mg/kg in sheep.

	Animal numbers							
Time (h)	143	144	145	146	147	148	149	150
2	UD	UD	UD	2.73	UD	UD	UD	UD
4	UD	UD	UD	47.27	UD	1.36	UD	UD
8	20.49	UD	2.67	80.94	UD	35.43	4.57	UD
12	106.70	UD	39.26	52.17	UD	82.12	26.79	UD
24	22.22	UD	12.97		UD	41.80	UD	UD
32	23.39	UD	4.24	12.66	UD	27.42	UD	2.80
48	1.13	UD	UD	1.65	UD	1.84	UD	UD
72	UD	UD	UD	UD	UD	UD	UD	UD
96	UD	UD	UD	UD	UD	UD	UD	
120	UD	UD	UD	UD	UD	1.76		
144	1.17	UD	UD	UD	UD	UD	UD	UD

UD = undetectable (<0.8 ng/ml)

Table B-13.
Concentrations of exudate prostaglandin E_2 (ng/ml) following intravenous administration of R(-) carprofen at 2.0 mg/kg in sheep.

	Animal numbers							
Time (h)	143	144	145	146	147	148	149	150
2	UD	UD	UD	2.12	UD	UD	6.79	10.15
4	99.11	36.36	UD	74.99	4.28	30.43	84.44	17.70
8	129.17	88.98	90.59	109.74	53.56	89.32	109.35	19.56
12	138.34	107.29	79.43	92.45	90.40	85.70	118.05	
24	128.58	86.88	56.30	59.35	27.93	52.41	107.83	21.72
32	124.97	74.02	16.04	35.36	59.81	1.41	37.54	
48	69.92	7.49	UD	23.32	11.10	1.54	6.80	41.42
72	11.34	UD	UD	7.22	UD	UD	1.23	39.40
96	6.10	2.36	UD	6.26	UD		1.93	49.41
120	1.99	UD			UD	UD	1.70	
144	1.88	UD	UD	6.15	UD	1.68	5.64	59.35

UD = undetectable (<0.8 ng/ml)

Table B-14.
Concentrations of exudate prostaglandin E_2 (ng/ml) following intravenous administration of S(+) carprofen at 2.0 mg/kg in sheep.

	Animal numbers							
Time (h)	143	144	145	146	147	148	149	150
2	UD	UD	UD	UD	UD	UD	UD	UD
4	UD	UD	6.05	16.20	UD	UD	UD	97.82
8	11.72	8.31	114.67	11.35	UD	2.81	7.80	UD
12	57.66	42.17	130.64	33.04	UD	29.76	12.04	12.07
24	7.33	12.73	81.07	7.07	UD	28.30	2.51	36.30
32	7.23	14.21	38.97		UD	53.44	UD	24.66
48	2.56	UD	2.79	2.42	UD	UD	UD	UD
72	UD	UD	UD	1.76	UD	UD	UD	UD
96	UD		2.16	2.06		UD		UD
120		UD	1.94			UD	UD	4.52
144		UD	2.11	UD	UD		UD	UD

UD = undetectable (<0.8 ng/ml)

Table B-15.
Concentrations of exudate prostaglandin E_2 (ng/ml) following intravenous administration of N^G-nitro-L-arginine methyl ester at 25 mg/kg in sheep.

Time (h)	Animal numbers							
	143	144	145	146	147	148	149	150
2	UD	UD	UD	16.02	UD	UD	UD	23.60
4	31.51	13.54	90.85	80.54	6.48	9.12	25.03	16.64
8	144.60	94.46	150.79	103.49	69.11	102.75	99.08	35.83
12	156.07	132.48	148.99	105.36	75.26	121.38	114.08	47.21
24	153.52	114.40	42.16	71.24	48.21	98.62	87.87	48.51
32	143.52	59.84	58.99	57.08		70.63	68.90	
48	98.21	4.56	4.77	43.08	UD	9.74	4.35	36.42
72	7.16	UD	UD	27.29	UD	1.56	UD	37.83
96		UD		21.59		1.27	UD	
120		UD		26.77			UD	
144	UD	UD	UD	26.67	UD	UD	UD	46.02

UD = undetectable (<0.8 ng/ml)

Table B-16.
Concentrations of exudate leukotriene B_4 (ng/ml) following intravenous administration of placebo in sheep.

Time (h)	Animal numbers							
	143	144	145	146	147	148	149	150
2	0.45	0.55	UD	UD	0.60	0.54	0.55	0.57
4	0.73	1.21	0.49	0.79	1.26	0.51	0.85	0.98
8	0.82	0.96	0.89	0.91	1.62	1.06	2.35	1.66
12	0.81	1.68	1.27	1.48	1.81	4.51	2.50	0.60
24	0.59	1.44	0.97	0.83	0.83	1.28	1.84	0.73
32	0.67	1.09	1.23	0.76	1.55		1.45	1.74
48		2.24	0.86	0.88	1.65	1.03	1.39	1.25
72	0.99	1.30	0.56	0.64	2.16	UD	1.74	1.16
96	0.78		1.71		UD	1.07	1.20	0.63
120	0.70		0.68		1.36			1.10
144	0.89	1.02	1.01	0.48	0.81	1.08	0.88	0.44

UD = undetectable (<0.16 ng/ml)

Table B-17.
Concentrations of exudate leukotriene B_4 (ng/ml) following intravenous administration of racemic carprofen at 4.0 mg/kg in sheep.

	Animal numbers							
Time (h)	143	144	145	146	147	148	149	150
2	0.68	1.61	0.91		0.59	0.60	0.40	0.70
4	1.03	0.61	1.23	1.21	0.42	0.82	0.67	1.12
8	1.22	1.05	1.24	0.87	0.93	1.01	1.20	0.87
12	1.82	1.71	3.92	1.07	1.12	1.45	2.59	1.35
24	0.56	0.87	1.79			1.00	1.41	0.91
32	0.95	0.79	2.50	0.93	2.00	1.01	1.94	1.26
48	1.20	0.94	1.64	1.54	1.03	0.52	1.49	1.75
72	0.81	1.28	1.35	1.69	1.17	1.02	1.69	1.02
96	0.75	0.80	1.39	1.61		1.09		0.70
120	1.10			0.86	1.54	0.63		
144	0.89	0.89	1.41	0.91	1.90	1.14	1.87	1.10

Table B-18.
Concentrations of exudate leukotriene B_4 (ng/ml) following intravenous administration of R(-) carprofen at 2.0 mg/kg in sheep.

	Animal numbers							
Time (h)	143	144	145	146	147	148	149	150
2	0.33	0.67	0.49	0.40	0.29	0.32	0.94	0.75
4	0.00	1.14	0.52	1.76	0.54	0.69	1.12	0.98
8	0.55	1.16	0.56	0.33	0.76	0.88	2.36	1.43
12	1.02	1.13	0.53	2.86	0.87	0.92	2.12	
24	0.75	1.05		0.68	0.51	0.91	0.92	1.99
32	0.53	1.09	0.83	2.40	1.03	1.63	1.18	
48	1.29	1.02	0.78	1.19	0.76	0.86	0.35	2.68
72	1.74	0.61	0.58	1.02	1.05	1.03	0.78	0.31
96	1.44	0.75	0.67	1.29	0.71		0.88	2.25
120	4.76	0.87	0.94		0.93	0.80	0.58	
144	1.04	1.83	0.56	2.64	0.75	0.88	0.78	1.18

Table B-19.
Concentrations of exudate leukotriene B_4 (ng/ml) following intravenous administration of S(+) carprofen at 2.0 mg/kg in sheep.

	Animal numbers							
Time (h)	143	144	145	146	147	148	149	150
2	UD	UD	0.92	0.53	0.58	0.58	0.63	0.90
4	0.96	0.37	0.84	1.68	1.10	0.90	1.20	2.06
8	0.56	0.73	2.06	1.22	2.95	1.60	1.99	2.83
12	0.92	1.53	2.27	8.51	2.00	0.98	3.68	2.94
24	0.52	1.10	1.99	1.44	1.38	0.93	1.18	1.95
32	0.54	2.53	1.31		1.22	0.80	2.54	1.94
48	0.52	0.44	4.61	0.95	8.98	1.54	1.60	3.01
72	6.55	0.62	2.13	1.74	5.21	0.87	1.33	3.17
96			2.39	1.55		1.17		1.25
120	0.97	0.48	3.88			0.94	1.29	0.77
144	0.27	0.72	1.25	2.14	0.99			0.83

Table B-20.
Concentrations of exudate leukotriene B_4 (ng/ml) following intravenous administration of N^G-nitro-L-arginine methyl ester at 25 mg/kg in sheep.

	Animal numbers							
Time (h)	143	144	145	146	147	148	149	150
2	0.57	0.43	0.62	0.94	0.38	UD	0.42	1.89
4	0.67	1.57	0.56	1.42	0.30	0.40	0.57	2.07
8	1.04	0.97	0.73	1.33	0.99	0.88	0.97	2.62
12	1.36	1.33	0.91	2.26	1.65	1.72	2.51	8.87
24	1.35	1.25	0.64	1.19	0.88	1.12	1.76	4.49
32	1.33	1.14	0.96	2.01		1.23	1.86	
48	1.42	0.96	0.90	1.39	0.70	1.18	2.26	3.19
72	1.11	1.25	1.73	1.23	0.69	1.06	1.18	3.15
96		0.78		1.60		1.14	3.55	
120		0.69		1.60			1.27	
144	0.86	0.63	0.89	1.88	0.92	0.82	1.29	2.57

UD = undetectable (<0.16 ng/ml)

Table B-21.
Numbers of exudate leukocytes (× 10^9 cells/L) following intravenous administration of placebo in sheep.

	Animal numbers							
Time (h)	143	144	145	146	147	148	149	150
4	2.4	2.5	3.6	5.3	9.1	4.9	10.2	4.8
8	4.1	4.9	8.8	4.5	0.0	12.1	6.8	2.7
12	8.3	10.5	13.1	9.1	32.6	26.2	9.9	31.3
24	14.9	5.6	11.1	17.0	38.1	8.1	6.9	34.5
48	4.2	3.9	9.1	4.2	8.8	3.6	5.0	4.0

Table B-22.
Numbers of exudate leukocytes (× 10^9 cells/L) following intravenous administration of racemic carprofen at 4.0 mg/kg in sheep.

	Animal numbers							
Time (h)	143	144	145	146	147	148	149	150
4	2.9	0.9	6.2	2.6	2.5	5.5	3.5	7.5
8	6.2	1.7	5.9	4.4	2.0	27.1	10.3	10.4
12	0.0	4.2	13.4	15.0	6.1	29.0	12.9	16.8
24	6.5	33.6	20.3	29.2	13.9	64.2	23.0	17.2
48	2.9	4.6	10.5	2.4	7.4	16.2	7.5	3.5

Table B-23.
Numbers of exudate leukocytes (× 10^9 cells/L) following intravenous administration of R(-) carprofen at 2.0 mg/kg in sheep.

	Animal numbers							
Time (h)	143	144	145	146	147	148	149	150
4	2.1	3.2	7.9	15.3	3.6	4.4	5.0	
8	5.7	5.8	10.6	2.1	5.5	6.0	19.3	5.4
12	9.3	0.0	12.3	15.4	13.7	20.2	27.8	
24	10.0	11.4	23.3	23.7	12.3	26.6	46.0	
48	11.3	8.6	4.8	16.7	11.7	7.9		

Table B-24.
Numbers of exudate leukocytes (× 10^9 cells/L) following intravenous administration of S(+) carprofen at 2.0 mg/kg in sheep.

	Animal numbers							
Time (h)	143	144	145	146	147	148	149	150
4	7.9	5.9	7.2	5.2	6.4	4.0	10.6	2.6
8	11.8	6.5	6.6	10.7	4.5	2.7	8.7	1.0
12	16.7	14.4	28.5	22.3	15.6	5.4	13.5	8.8
24	21.6	10.4	35.0	19.4	18.2	31.4	12.2	33.7
48	4.7	4.4	4.6	13.2	5.8	7.3	10.2	8.1

Table B-25.
Numbers of exudate leukocytes ($\times 10^9$ cells/L) following intravenous administration of N^G-nitro-L-arginine methyl ester at 25 mg/kg in sheep.

	Animal numbers							
Time (h)	143	144	145	146	147	148	149	150
4	3.8	5.3	5.3	11.7	3.9	4.7	2.9	14.6
8	5.7	3.8	12.8	27.9	3.3	15.6	7.1	3.6
12	6.6	6.9	21.0	49.2	8.3	25.1	10.6	
24	13.3	40.7	45.2	73.2	14.9	68.6	6.4	
48	3.4	5.0	4.1	10.9	15.0	9.1	0.0	10.8

Table B-26.
Numbers of leukocytes ($\times 10^9$ cells/L) in venous blood following intravenous administration of placebo in sheep.

	Animal numbers							
Time (h)	143	144	145	146	147	148	149	150
-0.33	6.0	6.4	9.1	5.8	7.2	6.4	11.1	9.4
4	6.1	5.9	8.2		8.0	5.6	10.2	9.0
24	6.6	6.4	7.8	5.6	8.9	6.5	9.7	9.9
48	6.1	6.1	8.4	3.9	8.8	6.0	6.9	8.5

Table B-27.
Numbers of leukocytes ($\times 10^9$ cells/L) in venous blood following intravenous administration of racemic carprofen at 4.0 mg/kg in sheep.

	Animal numbers							
Time (h)	143	144	145	146	147	148	149	150
-0.33	7.0	5.2	8.8	7.5	8.0	7.9	9.1	9.2
4	7.4	4.9	6.5	7.1		8.1	8.7	8.6
24	6.3	4.8	8.9	7.4	7.9	7.5	6.9	8.9
48	6.7	5.6	7.5	6.0	9.7	7.1	6.6	7.9

Table B-28.
Numbers of leukocytes ($\times 10^9$ cells/L) in venous blood following intravenous administration of R(-) carprofen at 2.0 mg/kg in sheep.

	Animal numbers							
Time (h)	143	144	145	146	147	148	149	150
-0.33	8.3	6.3	6.6	5.5	10.2	6.4	7.7	6.9
4	7.3		6.1	5.1	9.2		9.6	6.3
24	8.6	6.0	6.4	5.2	9.7	6.9	5.6	7.2
48	7.7	7.4	5.8	4.9	9.4	6.0	9.3	7.5

Table B-29.
Numbers of leukocytes (× 10^9 cells/L) in venous blood following intravenous administration of S(+) carprofen at 2.0 mg/kg in sheep.

	Animal numbers							
Time (h)	143	144	145	146	147	148	149	150
-0.33	7	5.5	7.1	4.7	9.9	9.9	7.7	8.8
4	7.1		8.0	4.3	5.8	9.1		10.2
24	6.6	5.8	7.5	4.0	2.8	9.5	8.0	8.7
48	6.4	5.6	6.6	3.9	6.2	8.0	6.6	8.3

Table B-30.
Numbers of leukocytes (× 10^9 cells/L) in venous blood following intravenous administration of N^G-nitro-L-arginine methyl ester at 25 mg/kg in sheep.

	Animal numbers							
Time (h)	143	144	145	146	147	148	149	150
-0.33	6.2	7.2	0.0	5.9		6.6	9.0	7.2
4		6.1		6.7		7.3	10.1	
24	9.1	7.8	7.4	7.6		8.7	10.2	7.1
48	6.1	6.8	5.8	6.1		6.5	11.0	7.5

Table B-31.
Numbers of platelets (× 10^9 cells/L) in venous blood following intravenous administration of placebo in sheep.

	Animal numbers							
Time (h)	143	144	145	146	147	148	149	150
-0.33	195	203	124	199	262	184	120	146
4	197	228	218		264	185	165	110
24	227	245	246	174	260	198	99	101
48	234	248	246	177	238	213	99	155

Table B-32.
Numbers of platelets (× 10^9 cells/L) in venous blood following intravenous administration of racemic carprofen at 4.0 mg/kg in sheep.

	Animal numbers							
Time (h)	143	144	145	146	147	148	149	150
-0.33	215	225	209	218	209	241	79	147
4	221	205	128	183		224	117	102
24	215	212	205	192	234	181	160	111
48	223	252	199	219	240	234	231	105

Table B-33.
Numbers of platelets ($\times 10^9$ cells/L) in venous blood following intravenous administration of R(-) carprofen at 2.0 mg/kg in sheep.

	Animal numbers							
Time (h)	143	144	145	146	147	148	149	150
-0.33	179	177	152	152	255	232	105	191
4	162		112	143	250		130	174
24	172	241	194	156	244	223	53	228
48	221	192	234	154	238	221	81	232

Table B-34.
Numbers of platelets ($\times 10^9$ cells/L) in venous blood following intravenous administration of S(+) carprofen at 2.0 mg/kg in sheep.

	Animal numbers							
Time (h)	143	144	145	146	147	148	149	150
-0.33	167	265	202	176	265	234	118	153
4	193		184	165	146	241		210
24	208	208	242	195	183	252	118	146
48	231	258	243	242	160	262	145	126

Table B-35.
Numbers of platelets ($\times 10^9$ cells/L) in venous blood following intravenous administration of N^G-nitro-L-arginine methyl ester at 25 mg/kg in sheep.

	Animal numbers							
Time (h)	143	144	145	146	147	148	149	150
-0.33	227	228	0	179	227	205	103	191
4		217		167	214	156	103	
24	186	243	195	159	256	192	99	187
48	202	239	204	217	258	188	103	162

Table B-36.
Concentrations of nitrite in exudate following intravenous administration of placebo in sheep (Values are expressed as μM of NO_2^- and NO_3^- was reduced into NO_2^-).

	Animal numbers							
Time(h)	143	144	145	146	147	148	149	150
-0.33	3.34	3.22	2.86	2.74	1.07	1.67	1.91	3.69
2	10.24	8.96	8.32	3.09	3.93	3.35	4.27	4.20
4	8.80	8.00	7.36	3.51	4.35	3.18	2.93	3.35
8	7.04	7.04	6.72	4.52	4.77	3.51	4.10	3.51
12	7.68	6.40	7.04	3.18	5.02	3.43	3.51	4.02
24	7.04	4.32	6.72	2.68	5.35	5.02	3.35	2.84
48	9.60	6.72	6.72	3.01	4.43	3.01	3.26	3.26
72				3.18	5.35	5.19	2.51	2.84
96				2.76	4.94	7.19	2.34	3.18

Table B-37.
Concentrations of nitrite in exudate following intravenous administration of N^G-nitro-L-arginine methyl ester in sheep (Values are expressed as μM of NO_2^- and NO_3^- was reduced into NO_2^-).

	Animal numbers							
Time(h)	143	144	145	146	147	148	149	150
-0.33	3.34	3.22	2.86	2.74	1.07	1.67	1.91	3.69
2	6.72	7.17	6.72	6.94	5.60	6.72	6.05	10.30
4	5.38	4.70	7.84	5.38	4.93	6.72	3.02	5.82
8	5.38	4.70	5.82	8.29	5.15	5.38	6.27	5.82
12	4.70	4.48	4.03	4.26	2.69	2.91	3.14	11.20
24	3.81	2.91	2.69	4.26	2.69	3.58	4.26	7.39
48	4.03	3.14	3.58	4.93	6.27	4.48	3.14	7.62
72	5.38	4.48	3.14	4.70	5.38	4.03	4.93	6.27
96	5.82	4.03	3.58	7.17	4.70	3.81	3.14	7.84

Appendix C

Pharmacokinetics and pharmacodynamics of phenylbutazone and flunixin meglumine in donkeys

Table C-1.
Concentrations (μg/ml) of phenylbutazone (PBZ), oxyphenbutazone (OPBZ) and flunixin meglumine (FM) in plasma following intravenous administration of PBZ (4.4 mg/kg), FM (1.1 mg/kg) in donkeys.

	Phenylbutazone			Oxyphenbutazone			Flunixin meglumine		
Time (h)	Cheeky	Frisky	Pinky	Cheeky	Frisky	Pinky	Cheeky	Frisky	Pinky
0.08	39.16	34.61	37.18	1.32	1.75	2.35	18.09	19.95	18.23
0.25	19.58	20.92	18.12	2.37	3.50	3.92	12.41	17.66	14.12
0.50	10.09	14.87	9.31	3.03	4.70	4.13	7.60	10.80	8.33
0.75	7.14	8.32	5.50	2.92	4.61	3.76	6.78	7.85	5.43
1	5.37	6.43	3.31	3.06	4.98	2.88	4.87	6.83	4.82
2	2.34	2.13	1.40	2.22	3.17	2.38	2.88	3.80	1.87
3	1.32	1.06	0.62	1.94	2.00	1.39	1.64	3.24	1.08
4	0.69	0.56	0.19	0.89	1.48	0.79	1.15	1.55	0.62
6	0.26	0.18	0.06	0.49	0.50	0.37	0.51	1.22	0.25
8	0.16	0.09	UD	0.38	0.29	UD	0.26	0.74	0.15
12	UD	UD	UD	0.11	0.09	UD	0.13	0.48	0.07
24	UD	UD	UD	UD	UD	UD	0.07	0.12	UD
32	UD	UD	UD	UD	UD	UD	UD	UD	UD

UD = undetectable (<0.05 μg/ml).

Table C-2.
Concentrations (μg/ml) of phenylbutazone (PBZ), oxyphenbutazone (OPBZ) and flunixin meglumine (FM) in exudate following intravenous administration of PBZ (4.4 mg/kg), FM (1.1 mg/kg) in donkeys.

	Phenylbutazone			Oxyphenbutazone			Flunixin meglumine		
Time (h)	Cheeky	Frisky	Pinky	Cheeky	Frisky	Pinky	Cheeky	Frisky	Pinky
2	0.20	2.33	1.67	0.08	1.26	0.58	1.09	1.94	0.77
4	0.38	0.97	0.75	0.25	1.32	0.58	0.87	1.21	0.88
8		0.53	0.33		0.67	0.34	0.49	0.36	0.37
12	0.43	0.14	0.06	0.21	0.20	0.14		UD	0.21
24	0.14	UD	0.05	0.06	UD	0.04	0.15	UD	
32	0.09	UD	UD	UD	UD	UD	0.15	UD	UD
48	0.07	UD	UD	UD	UD	UD	UD	UD	UD

UD = undetectable (<0.05 μg/ml).

Table C-3.
Concentrations (µg/ml) of phenylbutazone (PBZ), oxyphenbutazone (OPBZ) and flunixin meglumine (FM) in transudate following intravenous administration of PBZ (4.4 mg/kg), FM (1.1 mg/kg) in donkeys.

	Phenylbutazone			Oxyphenbutazone			Flunixin meglumine		
Time(h)	Cheeky	Frisky	Pinky	Cheeky	Frisky	Pinky	Cheeky	Frisky	Pinky
2	0.37	1.28	1.66	0.12	1.02	0.58	0.17	0.27	1.48
4	0.77	0.90	0.44	0.84	1.12	0.47	0.55	1.13	0.48
8		0.41	0.32		0.71	0.36	0.10	1.10	0.34
12	0.13	0.13	0.05	0.14	0.20	0.13	0.60	1.06	0.09
24	0.06	UD	0.05	UD	UD	UD	0.08	0.12	0.05
32	0.07	UD	UD	UD	UD	UD	0.08	0.27	0.05
48	0.07	UD	UD	UD	UD	UD	0.15	0.17	0.05

UD = undetectable (<0.05 µg/ml).

Table C-4.
Skin temperature (°C) over cages following intravenous administration of placebo, phenylbutazone (4.4 mg/kg) and flunixin meglumine (1.1 mg/kg) in donkeys (Values are expressed as the difference between inflamed and non-inflamed tissue-cages).

	Placebo			Phenylbutazone			Flunixin meglumine		
Time (h)	Cheeky	Frisky	Pinky	Cheeky	Frisky	Pinky	Cheeky	Frisky	Pinky
--0.33	-0.1	0.9	0.0	2.5	-0.9	0.2		-0.4	-0.9
1	0.3	1.0	0.2	0.0	-0.8	0.1	0.1	-0.1	-1.4
2	0.4	0.4	-1.2	-0.2	-0.4	0.2	0.3	0.3	-1.5
3	1.3	1.4	2.0	0.1	0.5	0.2	0.6	1.2	-1.5
4	3.7	0.4	0.3	1.0	-0.1	0.0	-0.3	0.5	-1.1
6	1.1	0.6	-0.5	0.3	-0.7	-0.3	1.0	0.4	-0.4
8	0.9	0.3	-0.1	-0.7	0.0	0.6	1.0	1.2	0.1
12	1.1	0.0	0.5	-0.2	0.0	1.8	0.4	1.3	1.0
24	2.2	1.2	1.1	1.7	0.9	0.1	1.3	-1.0	1.5
32	-2.3	1.0	0.5	2.7	0.0	1.0	0.3	-0.4	1.6
48	4.5	1.1	0.3	2.2	1.8	1.1	0.3	0.0	3.9

Table C-5.
Concentrations of serum thromboxane B_2 (ng/ml) following intravenous administration of placebo, phenylbutazone (4.4 mg/kg) and flunixin meglumine (1.1 mg/kg) in donkeys.

	Placebo			Phenylbutazone			Flunixin meglumine		
Time (h)	Cheeky	Frisky	Pinky	Cheeky	Frisky	Pinky	Cheeky	Frisky	Pinky
-0.33	46.50	90.50	34.80	41.05	41.50	32.60	21.65	80.50	28.55
1	31.70	87.00	35.10	4.90	14.40	3.20	5.80	0.00	1.55
2	43.80	78.00	27.20	6.25	39.25	7.25	3.60	7.30	0.00
4	45.05	89.00	32.70	15.75	90.50	20.80	4.00	9.00	2.15
6	43.80	70.00	32.50	17.35	60.00	23.20	3.65	0.00	2.65
8	47.05	74.00	32.70	42.20	53.00	33.20	6.10	2.25	9.05
12	37.80	86.00	31.20	28.55	75.00	32.20	8.50	11.65	9.90
24	36.00	62.00	26.50	44.33	66.00	26.00	26.60	34.20	32.60
32	35.25	64.00	28.35	38.30	57.00	30.90	29.65	92.50	31.45
48	36.60	48.00	23.40	51.00	61.00	29.65	32.65	61.00	33.35

Table C-6.
Concentrations of exudate prostaglandin E_2 (ng/ml) following intravenous administration of placebo, phenylbutazone (4.4 mg/kg) and flunixin meglumine (1.1 mg/kg) in donkeys.

	Placebo			Phenylbutazone			Flunixin meglumine		
Time (h)	Cheeky	Frisky	Pinky	Cheeky	Frisky	Pinky	Cheeky	Frisky	Pinky
2	0.17	0.19	1.19		UD	UD	UD	UD	UD
4	2.83	1.68	1.13	UD	UD	0.03	UD	UD	UD
8	4.23	1.26	1.19		UD	1.42	UD	UD	UD
12	4.48	2.52	2.99	3.36	2.38	2.99	UD	3.56	2.00
24	4.48	0.13		3.36	2.83	2.25	UD	UD	3.56
32	2.83		3.36	3.99	1.78	1.78	UD		3.56
48	1.50		1.13	1.19	0.63	0.19	UD		3.76

UD = undetectable (<0.8 ng/ml).

Table C-7.
Concentrations of exudate 12-hydroxyeicosatetraenoic acid (ng/ml) following intravenous administration of placebo, phenylbutazone (4.4 mg/kg) and flunixin meglumine (1.1 mg/kg) in donkeys.

	Placebo			Phenylbutazone			Flunixin meglumine		
Time (h)	Cheeky	Frisky	Pinky	Cheeky	Frisky	Pinky	Cheeky	Frisky	Pinky
2	394.87	580.37	380.36	252.30	407.43	593.56	528.94	212.83	485.43
4	495.96	900.85	562.16	230.36	605.29	613.70	558.62	610.75	517.58
8	527.65	939.64			364.80	651.52	859.61		663.12
12	420.74	428.05	350.08	491.86	339.33	633.55	392.39	400.39	589.34
24	282.62	452.62		283.06	451.09	275.08	364.97		569.75
32	274.39	689.32		293.46	389.80	348.13	355.77		639.02
48	328.70	986.35	446.41	272.74	463.34	301.21	357.20		738.96

Table C-8.
Numbers of exudate leukocytes (× 10^9 cell/L) following intravenous administration of placebo, phenylbutazone (4.4 mg/kg) and flunixin meglumine (1.1 mg/kg) in donkeys.

	Placebo			Phenylbutazone			Flunixin meglumine		
Time (h)	Cheeky	Frisky	Pinky	Cheeky	Frisky	Pinky	Cheeky	Frisky	Pinky
2	6.90	6.90			3.40	0.70	41.80		2.10
4	5.60	2.60			2.10	1.20			0.70
8	7.90	0.50	2.20	3.10	2.80	3.70	1.70	14.10	2.60
12	44.10	12.40	3.20	14.10	42.40	1.40	21.60	4.90	
24	29.30	7.20		17.50	8.80	18.40	33.30		3.90
32	11.20	1.70	2.70	13.50	5.20	9.30	14.80		1.60
48	7.70	3.50	2.10	5.10	3.80	3.90	4.70	2.20	2.60

Table C-9.
Numbers (× 10^9 cell/L) of leukocytes in venous blood following intravenous administration of placebo, phenylbutazone (4.4 mg/kg) and flunixin meglumine (1.1 mg/kg) in donkeys.

	Placebo			Phenylbutazone			Flunixin meglumine		
Time (h)	Cheeky	Frisky	Pinky	Cheeky	Frisky	Pinky	Cheeky	Frisky	Pinky
-0.33	9.50	9.70	5.10	10.50	9.00	4.60	9.00	11.00	4.70
6	9.10	8.40	4.80	9.90	8.50	4.60	9.60	10.20	5.50
24	10.70	9.60	6.10	10.20	8.20	5.60	10.60		5.10
32	9.70	11.10	6.00	10.50	8.50	6.80	11.00	11.10	5.80
48	9.00	11.70			7.70	5.50	10.10		5.50

Table C-10.
Numbers (× 10^9 cell/L) of platelets in venous blood following intravenous administration of placebo, phenylbutazone (4.4 mg/kg) and flunixin meglumine (1.1 mg/kg) in donkeys.

	Placebo			Phenylbutazone			Flunixin meglumine		
Time (h)	Cheeky	Frisky	Pinky	Cheeky	Frisky	Pinky	Cheeky	Frisky	Pinky
-0.33	144.00	154.00	88.00	111.00	206.00	95.00	136.00	146.00	120.00
6	144.00	191.00	110.00	130.00	229.00	107.00	138.00	202.00	134.00
24	152.00	180.00	111.00	108.00	216.00	117.00	144.00		126.00
32	144.00	156.00	101.00	112.00	146.00	84.00	136.00		100.00
48	145.00	179.00			193.00	107.00	121.00		126.00

Appendix D

Experimental code: QM/ZC/08/94

Study on pharmacokinetics, bioavailability and serum thromboxane B_2 inhibition of phenylbutazone in goats

Table D-1.
Concentrations of phenylbutazone (PBZ) in plasma following PBZ administered orally at 4.4 mg/kg in goats.

	Animal numbers					
Time (h)	G7	G10	G11	G13	G72	G74
Pre.	UD	UD	UD	UD	UD	UD
0.25	7.56	4.50		5.38	10.45	12.76
0.50	16.01	17.27	9.80	7.86	18.89	16.54
0.75	21.35	17.98	13.49	5.74	21.64	22.24
1	26.33	19.94	13.30	9.79	23.85	23.04
2	29.33	14.31	18.75	15.31	28.58	17.41
4	30.53	26.07	27.25	16.61	28.94	27.81
6	31.10	21.44	24.55	18.97	26.75	28.64
8	27.62	21.97	25.86	18.91	24.24	24.46
12	14.46	17.92	22.92	15.84	34.80	27.41
24	14.95	9.70	17.31	8.51	14.01	22.77
34		6.76	15.04	5.57	9.32	19.04
48		2.47	9.83	1.78	4.54	12.07
72	1.93	0.74	1.17		1.88	7.24
96			1.68			3.27
120			0.65			1.63
144			0.26			0.85

UD = undetectable (<0.05 μg/ml).

Table D-2.
Concentrations of oxyphenbutazone in plasma following phenylbutazone administered orally at 4.4 mg/kg in goats.

	Animal numbers					
Time (h)	G7	G10	G11	G13	G72	G74
Pre.	UD	UD	UD	UD	UD	UD
0.25	UD	UD	UD	UD	UD	UD
0.50	UD	0.09	UD	UD	0.08	UD
0.75	UD	0.14	0.07	UD	0.13	UD
1	0.07	0.20	0.17	UD	0.17	UD
2	0.15	0.22	0.35	0.17	0.32	0.10
4	0.25	0.65	0.45	0.25	0.47	0.11
6	0.26	0.62	0.54	0.50	0.66	0.14
8	0.22	0.79	0.63	0.18	0.11	0.33
12	0.27	1.12	0.48	0.47	0.41	0.20
24	0.16	0.32	0.30	0.18	0.36	0.18
34		0.28	0.26	UD	0.22	0.15
48		0.07	UD	UD	0.01	0.13
72	0.08	UD	UD	UD	UD	0.07
96			UD			UD
120			UD			UD
144			UD			UD

UD = undetectable (<0.05 μg/ml).

Table D-3.
Concentrations of phenylbutazone (PBZ) in plasma following PBZ administered intravenously at 4.4 mg/kg in goats.

	Animal numbers					
Time (h)	G7	G10	G11	G13	G72	G74
Pre.	UD	UD	UD	UD	UD	UD
0.03	93.99	124.59	170.87	69.92	131.62	146.39
0.08	86.29	127.40		59.20	132.97	
0.17	86.10	91.78	107.63	43.84	99.26	114.41
0.25	74.53	77.15	145.65	41.67	86.74	106.77
0.50	62.81	74.50	94.95	37.96	78.56	91.49
0.75	57.76	65.70	84.51	33.43	66.26	84.60
1	53.50	58.17	75.15	30.81	65.41	82.08
2	42.21	47.78	66.76	25.43	49.16	67.75
4	33.47	42.34	59.98	17.53	43.57	57.17
6	33.24	35.81	45.83	20.46	38.22	55.19
8	31.02	33.35	46.09	15.07	34.85	55.02
12	24.90	28.15	37.11	12.66	29.77	46.64
24	14.63	14.78	23.92	6.09	17.01	31.48
34	10.83	10.44	18.18	3.81	13.35	25.38
48	5.05	5.27	8.25	1.57	7.63	18.43
72	2.06	1.82	2.77	0.41	2.76	3.21
96		0.58			1.00	
120		0.22			0.29	
144		0.06			0.11	

Table D-4.
Concentrations of oxyphenbutazone in plasma following phenylbutazone administered intravenously at 4.4 mg/kg in goats.

Time (h)	Animal numbers					
	G7	G10	G11	G13	G72	G74
Pre.	UD	UD	UD	UD	UD	UD
0.03	UD	0.19	0.30	UD	UD	UD
0.08	0.10	0.42		0.17	0.38	UD
0.17	0.19	0.46	0.72	0.21	0.49	0.21
0.25	0.21	0.66	1.24	0.28	0.57	0.24
0.50	0.25	0.90	1.30	0.26	0.70	0.32
0.75	0.22	0.93	1.39	0.45	0.68	0.38
1	0.23	0.89	1.47	0.47	0.69	0.37
2	0.27	0.90	1.52	0.39	0.62	0.45
4	0.24	0.95	1.65	0.43	0.61	0.16
6	0.35	0.86	1.39	0.35	0.63	0.47
8	0.43	1.06	1.34	0.49	0.18	0.42
12	0.43	0.86	1.09	0.60	0.55	0.61
24	0.12	0.41	0.76	0.12	0.37	0.23
34	0.08	0.23	0.50	0.10	0.19	0.14
48	UD	0.09	0.33	UD	0.13	0.15
72	UD	UD	0.06	0.06	UD	UD
96		UD			UD	
120		UD			UD	
144		UD			UD	

UD = undetectable (<0.05 μg/ml).

Table D-5.
Concentrations of serum thromboxane B_2 (ng/ml) following placebo administered intravenously in goats.

	Animal numbers					
Time (h)	G7	G10	G11	G13	G72	G74
-0.08	96.92	274.98	119.75	137.77	281.14	149.78
1	21.41	125.19	13.86	158.20	205.24	58.19
2	18.20	128.30	12.67	52.03	204.49	41.11
4	154.47	105.85	10.13	62.42	221.64	35.35
6	149.78	116.90	33.48	53.65	187.82	40.93
8	210.37	151.74	20.00	77.26	209.61	45.00
12	134.05	129.44	18.04	61.14	185.60	55.26
24	272.94	186.68	18.60	125.42	290.55	69.27
32	208.12	151.34	23.15	110.19	224.49	67.33
48	153.64	188.82	43.08	108.84	246.90	209.68
72	180.47	208.89	49.47	117.32	264.43	100.73
96			108.71			200.14
120			42.50			197.40
144			31.77			170.47

Table D-6.
Concentrations of serum thromboxane B_2 (ng/ml) following phenylbutazone administered intravenously at 4.4 mg/kg in goats.

	Animal numbers					
Time (h)	G7	G10	G11	G13	G72	G74
-0.08	109.06	166.72	405.22	193.52	257.24	175.03
1	9.63	12.93	312.09	17.50	49.82	94.84
2	25.29	20.40	368.31	37.32	65.38	101.63
4	20.77	28.84	388.12	74.17	115.93	97.11
6	17.89	40.56	315.96	38.08	158.83	136.55
8	22.70	45.00	419.51	71.55	160.50	84.62
12	22.03	56.76	326.85	32.57	174.19	139.49
24	87.41	112.08	397.49	162.78	248.73	221.15
32	79.43	135.77	398.72	142.81	310.73	256.96
48	83.00	182.72	385.43	137.13	290.26	339.41
72	66.75	274.10	406.16	154.83	339.31	258.48
96		203.33			269.04	
120		234.28			356.71	
144		186.95			402.15	

Appendix E

Experimental Code: QM/ZC/10/94

Comparative pharmacokinetics of paracetamol (acetaminophen) and its sulphate and glucuronide metabolites in desert camels and goats.

Table E-1.
Concentrations of paracetamol in serum following intramuscular administration of paracetamol at 5 mg/kg in camels.

	Animal numbers					
Time (min)	C1	C2	C3	C4	C5	C6
Pre	0.00	0.00	0.00	0.00	0.00	0.00
10	3.18	7.34	3.10	3.46	1.27	3.40
20	3.53	6.88	3.40	3.98	1.65	3.19
30	4.11	5.63	3.35	4.15	1.72	2.72
40	3.83	5.63	2.90	3.71	1.90	2.47
60	3.39	3.91	2.30	3.44	1.40	1.64
90	2.43	2.96	1.73	2.15	1.28	1.18
120	1.33	1.58	1.19	1.23	0.95	0.68
150	0.75	1.02	0.68	0.89	0.65	0.43
180	0.53	0.65	0.53	0.59	0.44	0.86
210	0.35	0.33	0.41	0.35	0.38	0.19

Table E-2.
Concentrations of paracetamol β-glucuronide in serum following intramuscular administration of paracetamol at 5 mg/kg in camels.

	Animal numbers					
Time (min)	C1	C2	C3	C4	C5	C6
Pre	0.00	0.00	0.00	0.00	0.00	0.00
10	0.56	2.36	0.79	2.37	0.37	0.61
20	2.70	5.11	1.45	2.80	1.12	1.28
30	3.38	6.04	1.82	3.51	1.19	1.87
40	3.61	8.21	2.10	3.92	3.26	2.86
60	6.27	10.95	3.30	6.35	3.93	3.31
90	8.59	12.59	4.11	6.84	5.37	3.21
120	9.15	11.22	4.21	6.66	5.64	3.35
150	7.21	8.16	3.77	7.26	4.65	2.49
180	6.50	7.19	3.42	6.89	3.76	2.39
210	5.57	5.93	3.15	6.15	3.09	1.88

Table E-3.
Concentrations of paracetamol sulphate in serum following intramuscular administration of paracetamol at 5 mg/kg in camels.

	Animal numbers					
Time (min)	C1	C2	C3	C4	C5	C6
Pre	0.00	0.00	0.00	0.00	0.00	0.00
10	4.78	6.72	2.27	8.21	1.09	2.99
20	9.19	11.83	5.02	9.39	1.93	4.71
30	10.38	10.75	5.70	11.09	3.01	5.26
40	10.29	14.70	5.72	9.73	4.19	7.55
60	16.35	16.17	9.19	18.25	4.38	7.33
90	20.43	16.63	10.44	15.36	5.61	6.21
120	9.22	11.34	9.77	11.25	4.85	5.60
150	9.82	6.83	7.55	13.51	3.60	3.33
180	8.82	5.95	5.71	10.95	3.57	2.70
210	5.95	3.53	5.01	7.87	2.14	1.19

Table E-4.
Concentrations of paracetamol in serum following intravenous administration of paracetamol at 5 mg/kg in camels.

	Animal numbers					
Time (min)	C1	C2	C3	C4	C5	C6
Pre	0.00	0.12	0.00	0.00	0.00	0.00
5	8.44	14.16	8.81	10.32	7.13	missing
10	5.57	6.02	5.79	8.04	5.90	6.18
15	3.10	4.13	3.59	3.84	4.07	5.65
20	2.99	2.62	2.90	2.76	3.39	4.35
30	1.97	2.41	2.06	2.28	2.54	2.98
40	1.11	1.62	1.68	1.96	1.98	2.10
60	0.70	0.99	0.98	1.06	1.59	1.74
90	0.40	0.72	0.59	0.75	0.84	1.11
120	0.23	0.43	0.44	0.37	0.61	0.58
150	0.31	0.32	0.32	0.30	0.36	0.36
180	0.11	0.21	0.20	0.20	0.25	0.25

Table E-5.
Concentrations of paracetamol β-glucuronide in serum following intravenous administration of paracetamol at 5 mg/kg in camels.

	Animal numbers					
Time (min)	C1	C2	C3	C4	C5	C6
Pre	0.00	0.00	0.00	0.00	0.00	0.00
5	3.97	6.34	1.86	1.97	4.04	missing
10	5.79	9.94	4.11	5.74	6.89	5.92
15	6.99	11.25	5.65	6.79	7.17	8.21
20	7.58	13.18	5.73	6.88	8.26	8.10
30	7.34	15.29	6.59	6.96	8.81	7.33
40	6.80	10.71	6.65	7.02	10.25	7.38
60	6.25	9.91	5.45	6.75	10.60	7.38
90	5.60	9.33	4.57	5.82	7.95	6.76
120	4.95	8.83	3.99	4.28	6.59	7.17
150	4.63	7.15	3.42	4.85	6.00	4.49
180	3.28	5.69	2.48	3.88	4.97	4.28

Table E-6.
Concentrations of paracetamol sulphate in serum following intravenous administration of paracetamol at 5 mg/kg in camels.

	Animal numbers					
Time (min)	C1	C2	C3	C4	C5	C6
Pre	0.00	0.00	0.00	0.00	0.00	0.00
5	10.52	8.07	6.41	7.76	9.95	missing
10	14.00	14.56	11.41	17.48	12.98	13.80
15	17.00	20.67	12.14	15.04	10.79	19.04
20	20.18	15.60	15.00	15.00	12.50	20.75
30	15.25	13.77	13.64	17.14	12.34	17.21
40	11.66	7.89	13.29	11.68	12.04	16.86
60	6.20	5.53	9.09	10.22	11.73	17.74
90	5.53	5.26	6.42	7.31	8.16	11.83
120	3.23	3.91	4.98	4.26	6.01	10.89
150	3.27	2.95	3.59	4.91	4.88	5.56
180	1.75	1.99	2.26	3.32	2.67	4.13

Table E-7.
Concentrations of paracetamol in serum following intramusular administration of paracetamol at 10 mg/kg in goats.

Time (min)	Animal numbers				
	G1	G2	G3	G4	G5
Pre	0.00	0.00	0.00	0.00	0.00
10	4.91	5.77	4.98	4.76	2.78
20	3.90	4.79	4.69	4.38	2.86
30	2.44	2.66	3.02	3.94	2.91
45	1.38	1.48	1.28	3.24	2.23
60	0.69	0.60	0.64	1.91	1.43
90	0.25	0.10	0.14	0.64	missing
120	0.10	UD	0.09	0.25	0.37
150	UD	UD	UD	0.12	0.17
240	UD	UD	UD	0.09	142.11

UD = undetectable (<0.1 μg/ml).

Table E-8.
Concentrations of paracetamol β-glucuronide in serum following intramusular administration of paracetamol at 10 mg/kg in goats.

Time (min)	Animal numbers				
	G1	G2	G3	G4	G5
Pre	0.00	0.00	0.00	0.00	0.00
10	20.24	19.49	21.24	12.53	8.19
20	45.24	44.58	44.56	25.33	13.52
30	56.41	47.39	54.31	31.66	19.32
45	48.33	39.06	44.91	38.64	25.89
60	38.75	29.74	34.82	36.21	25.49
90	21.93	16.63	20.19	20.62	missing
120	12.99	8.41	10.44	9.60	17.47
150	8.43	6.02	6.79	5.98	7.28
240	2.83	1.89	2.75	1.52	5.04

Table E-9.
Concentrations of paracetamol sulphate in serum following intramusular administration of paracetamol at 10 mg/kg in goats.

	Animal numbers				
Time (min)	G1	G2	G3	G4	G5
Pre	0.00	0.45	0.00	0.05	0.00
10	0.66	0.64	0.31	0.98	0.14
20	1.34	1.80	0.85	2.12	0.56
30	1.66	1.57	1.16	2.29	0.37
45	2.60	1.80	1.03	2.94	0.71
60	1.05	0.90	0.57	2.64	0.45
90	0.70	0.51	0.29	1.32	missing
120	0.23	0.28	0.10	0.42	missing
150	UD	0.42	0.10	0.28	0.17
240	UD	0.18	UD	0.10	UD

UD = undetectable (<0.1 μg/ml).

Table E-10.
Concentrations of paracetamol in serum following intravenous administration of paracetamol at 10 mg/kg in goats.

	Animal numbers					
Time (min)	G1	G2	G3	G4	G5	G6
Pre	0.00	0.00	0.00	0.00	0.00	0.00
5	11.96	8.03	13.37	13.09	8.63	8.63
10	5.13	6.39	9.85	6.65	6.76	6.76
20	2.75	3.28	6.88	4.18	5.67	5.67
30	1.71	2.37	4.11	2.07	3.64	3.64
40	0.76	1.13	2.54	1.10	2.07	2.07
60	0.29	0.35	0.94	0.44	1.01	1.01
90	0.10	0.10	0.27	missing	0.73	0.73
120	UD	UD	UD	0.13	0.24	0.24
150	UD	UD	UD	0.10	0.11	0.11
210	UD	UD	UD	UD	UD	UD

UD = undetectable (<0.1 μg/ml).

Table E-11.
Concentrations of paracetamol β-glucuronide in serum following intravenous administration of paracetamol at 10 mg/kg in goats.

	Animal numbers					
Time (min)	G1	G2	G3	G4	G5	G6
Pre	0.00	0.00	0.00	0.00	0.00	0.00
5.00	19.00	31.44	25.32	26.77	51.66	72.14
10.00	51.18	33.86	38.46	41.28	54.04	77.99
20.00	55.45	32.65	47.27	51.42	56.77	85.54
30.00	52.43	32.81	45.07	50.83	55.39	79.78
40.00	39.95	30.08	34.21	34.96	48.93	59.82
60.00	27.88	23.75	25.99	21.71	39.77	43.39
90.00	17.89	13.27	15.93	missing	28.32	33.91
120.00	11.37	9.40	7.01	8.04	18.74	13.61
150.00	7.16	4.98	4.21	6.80	9.79	8.32
210.00	3.18	2.03	2.60	2.31	6.44	4.16

Table E-12.
Concentrations of paracetamol sulphate in serum following intravenous administration of paracetamol at 10 mg/kg in goats.

	Animal numbers				
Time (min)	G1	G2	G3	G4	G5
Pre	0.00	0.00	0.00	0.00	0.00
5	1.21	1.10	2.09	0.81	1.52
10	2.91	0.68	2.75	2.08	1.57
20	2.36	0.57	3.57	2.08	1.78
30	2.86	0.63	3.95	2.00	1.50
40	1.56	0.44	2.54	1.14	1.36
60	1.65	0.27	1.63	0.84	1.19
90	0.71	0.24	0.54		0.67
120	1.04		0.39		0.24
150	0.31		0.13		0.10
210	0.79		0.14		UD

UD = undetectable (<0.1 μg/ml).