

Pharmacokinetics and Pharmacodynamic Effects of Flunixin after Intravenous, Intramuscular and Oral Administration to Dairy Goats

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¹Department of Obstetrics and Gynaecology, Centre for Reproductive Biology in Uppsala, and ²Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, Swedish University of Agricultural Sciences, Uppsala, Sweden, and ³Department of Reproduction and Forensic Medicine, Norwegian College of Veterinary Medicine, Oslo, Norway.

Königsson K, Törneke K, Engeland IV, Odensvik K, Kindahl H: Pharmacokinetics and pharmacodynamic effects of flunixin after intravenous, intramuscular and oral administration to dairy goats. Acta vet. scand. 2003, 44, 153-159. – The pharmacokinetics and the prostaglandin (PG) synthesis inhibiting effect of flunixin were determined in 6 Norwegian dairy goats. The dose was 2.2 mg/kg body weight administered by intravenous (i.v.), intramuscular (i.m.) and oral (p.o.) routes using a cross-over design. Plasma flunixin content was analysed by use of liquid chromatography and the PG synthesis was evaluated by measuring plasma 15-ketodihydro-PGF_{2α} by a radioimmuno-assay. Results are presented as median (range). The elimination half-lives ($t_{1/2\lambda}$) were 3.6 (2.0-5.0), 3.4 (2.6-6.8) and 4.3 (3.4-6.1) h for i.v., i.m. and p.o. administration, respectively. Volume of distribution at steady state ($V_{d_{ss}}$) was 0.35 (0.23-0.41) L/kg and clearance (CL), 110 (60-160) mL/h/kg. The plasma concentrations after oral administration showed a double-peak phenomenon with the two peaks occurring at 0.37 (0.25-1) and 3.5 (2.5-5.0) h, respectively. Both peaks were in the same order of magnitude. Bioavailability was 79 (53-112) and 58 (35%-120)% for i.m. and p.o. administration, respectively. 15-Ketodihydro-PGF_{2α} plasma concentrations decreased after flunixin administration independent of the route of administration.

Introduction

Flunixin is a non-steroid anti-inflammatory drug (NSAID) used for analgetic, antiphlogistic and antipyretic purposes in a variety of mammalian species. The mechanism of action of NSAIDs is inhibition of cyclooxygenase (COX), responsible for the synthesis of prostaglandins (PG:s) from arachidonic acid (Vane & Botting 1996).

Flunixin has been studied for treatment of inflammatory conditions like mastitis, endotoxemia and musculoskeletal disorders in different ruminant species (e.g. Anderson *et al.* 1991, Welsh & Nolan 1995, Odensvik & Magnusson

1996) and its pharmacokinetic properties has been studied in cattle (Landoni *et al.* 1995, Odensvik & Johansson 1995), sheep (Welsh *et al.* 1993, Cheng *et al.*, 1998), camels (Oukessou 1994, Wasfi *et al.* 1998) and llamas (Navarre *et al.* 2001).

For NSAIDs, the relationship between plasma concentration and clinical effect has been difficult to establish (Cheng *et al.* 1998). However, one biomarker for PG synthesis, which could be detected in plasma, is 15-ketodihydro-PGF_{2α} (PG-metabolite). This is the primary metabolite of PGF_{2α} and has been used in several studies as a marker for synthesis and re-

lease of PG:s (e.g. Fredriksson 1984, Daels et al. 1989).

The aim of this study was to investigate the pharmacokinetics of flunixin in Norwegian dairy goats after oral, intramuscular and intravenous administrations and to study the inhibition of prostaglandin synthesis.

Materials and methods

Animals

6 healthy Norwegian dairy goats were used for the study. The age of the goats was 3 years and the body weights ranged from 34 to 59 kg. The goats were fed according to Norwegian standards. The same 6 animals were used in all studies, i.e. single intravenous administration (i.v.), single intramuscular administration (i.m.) and single oral administration (p.o.). The experiments were carried out in the post partal period (9-61 days after parturition). Four of the goats were lactating and the other two goats had aborted and were non-lactating. The study has been registered and approved by the Norwegian Animal Research Authority (NARA) (Registration No. 5/97).

Drug administration

Flunixin meglumine (Finadyne[®] vet., Schering-Plough, Stockholm, Sweden) was administered in all experiments at a nominal dose of 2.2 mg/kg body weight. For i.v. administration, Finadyne[®] vet. solution for injection, (50 mg/mL) was given into the *V. jugularis externa*, and for i.m. administration the same formulation was given into the dorsal muscles of the neck. For oral administration Finadyne[®] vet granules (25 mg/g) dissolved in a small amount of water was given via a gastric tube as a gavage. All administrations were performed at 9 am.

Blood sampling

Before each experiment an intravenous catheter was inserted into the jugular vein (flunixin was

administered into the contralateral jugular vein). Blood samples (approximately 5 mL) were harvested at 60, 45, 30, 15 and 5 minutes before and at 5, 15, 30, 45, 60, 90, 120, 150 and 180 minutes and at 4, 5, 6, 8, 10, 12, 16, 22, 24, 26, 28, 30, 32, 34 and 36 h after administration of flunixin.

The blood was collected in heparinized tubes (Vacutainer, Terumo, Loeven, Belgium). Plasma was separated by centrifugation at 1500 × g, for 20 min at 20°C within 20 min after collection. Plasma was withdrawn and stored at -20°C until analysed.

Experimental design

The study had a crossover design and all the six participating animals received flunixin i.v., i.m. and p.o. Drug administration was carried out at three successive periods with a washout time of 10 days between each experiment. At each period, two animals received each of the administrations. The goats were randomly allocated to the administration groups.

Analytical assay

Flunixin was analysed using a high performance liquid chromatography (HPLC) method adapted from analysis of bovine plasma (Odensvik & Johansson, 1995). In short, goat plasma was used for analysis and as an internal standard, sodium diclofenac (Ciba-Geigy AG, Basel, Schweiz) dissolved in potassium phosphate buffer (KH₂PO₄) pH 3.5 (0.3 M) was used. For each sample 1 mL of goat plasma was used and the internal standard (200 µl) was added to the plasma and mixed. Then flunixin and diclofenac were extracted by an addition of 5 mL of diethyl ether. After gentle mixture for 20 min the tube with its contents were put in methanol, -20°C. When the plasma had frozen the ether-phase was removed and transferred to a second tube and then evaporated. The residue was dissolved in 200 µL mobile phase and

injected on the column (LiChrosorb 5 RP-SELECT-B). Methanol (LiChrosolve Methanol gradient grade for chromatography, Merck, Darmstadt, Germany) and sodium phosphate buffer pH 5.8 (0.05 M) 50/50 vol/vol were used as mobile phase. All chromatographic procedures were performed with a flow rate of 0.8 mL/min and a run time of 20 min. Flunixin was detected by UV absorption at a wavelength of 254 nm. The retention time for diclofenac and flunixin were 10 and 12 min, respectively. The standard curve ranged from 47 ng/mL to 29 µg/mL. The limit of quantification for the method was 47 ng/mL. At 47 ng/mL the intra-assay coefficient of variation and the inter-assay coefficient of variation were 7.8% (n=3) and 9.3% (n=6), respectively.

For each series of analysis a standard curve was generated and in addition 6 quality control samples (3 different concentrations) were analysed together with the test samples. As acceptance criterion for an analysis the quality control samples should have a precision and accuracy equal to or better than 10% of the intended concentration. Deviations of up to 20% were accepted for 2 of the 6 quality control samples unless both deviations occurred at the same concentration.

15-Ketodihydro-PGF_{2α} was analysed using a radioimmunoassay (Granström & Kindahl, 1982). The analyses were performed in duplicates of 0.5 ml plasma. Limit of quantification for the method was 30 pmol/L. Samples of higher concentrations were reanalysed in 0.2 ml (>200 pmol/L) or 0.05 ml (>500 pmol/L) plasma in separate assays. The range of the standard curve was 4-512 pg and then, depending on plasma volumes, the final concentrations were calculated in pmol/L. Intra-assay coefficients of variation ranged between 6.6% and 11.7% for the different ranges of the standard curve and the inter-assay coefficient of variation was 14%.

Pharmacokinetic calculations

Data for plasma flunixin concentration vs. time was analysed by use of non-compartmental methods based on statistical moment theory (Gibaldi & Perrier, 1982). A commercially available software program was used (WinNonlin Standard, Pharsight Corporation, Palo Alto, CA, USA) with its incorporated models (number 200 for im and po administration and 201 for iv administration). The area under the plasma concentration-vs.-time curve (AUC) and the area under the first moment curve (AUMC) was calculated using the linear/logarithmic trapezoidal rule. The rate constant associated with the terminal elimination phase (λ) was estimated by means of linear regression of the terminal phase of the log concentration-vs.-time curve, and the half-life ($t_{1/2}$) and the volume of distribution (Vd) associated with λ (Vd_λ) was calculated. For calculation of λ after the intravenous and the intramuscular administration all plasma samples from 2.5 h and onwards were included. After oral administration of the drug all time points from 6 h and further were included. Lambda was also used to extrapolate AUC and the AUMC to infinity (inf). From AUC and AUMC, clearance (CL), mean residence time (MRT) and volume of distribution at steady state (Vd_{ss}) were calculated.

The equations used for the calculations were:

$$F \text{ (bioavailability)} = 100 \times (AUC_{\text{inf,extravas.}} \times \text{dose}_{\text{iv}}) / (AUC_{\text{inf,iv}} \times \text{dose}_{\text{oral}})$$

$$Vd_\lambda = \text{Dose} / (\lambda \times AUC_{\text{inf}})$$

$$Vd_{ss} = \text{Dose} \times \text{AUMC} / AUC_{\text{inf}^2}$$

$$Cl = \text{dose} / AUC_{\text{inf}}$$

$$\text{MRT} = \text{AUMC}_{\text{inf}} / AUC_{\text{inf}}$$

Pharmacodynamic calculations

For the evaluation of the effect on prostaglandin release, a pre-experimental mean value of the PG-metabolite concentration was calculated for each goat at each administration. A cut-off limit

for PG-metabolite suppression was calculated and defined as the pre-experimental mean value minus two times the standard deviation. Inhibition of the prostaglandin synthesis was assumed to last as long as the PG-metabolite levels remained below the cut-off limit.

Statistical analyses

All pharmacokinetic parameters are expressed as median (range). Differences between different routes of administration regarding the λ and the effect duration were statistically evaluated using the Kruskal-Wallis test by the use of Minitab for Windows 95, release 12 (Minitab, State College, PA, USA). $P < 0.05$ was considered as the level of significance.

Results

Pharmacokinetic parameters

Flunixin was detected in the plasma samples irrespective of route of administration at all time points up to 22 (12-26) hours (median (range)). The plasma concentrations of flunixin after i.v. administration declined rapidly with two distinct phases prior to the λ -phase. The latter of

these assumed distribution phases was seen also after i.m. administration (Fig. 1).

Flunixin absorption after oral administration was rapid with two C_{\max} in all individuals (Fig 2). The second C_{\max} was of the same magnitude as the first. The terminal phase of the plasma concentration-time profiles had similar (Kruskal-Wallis, $p > 0.05$) elimination rates independent of route of administration (Fig. 1 and Fig. 2). The median (range) r^2 for the linear regression of the terminal phase of the curve was 0.98 (0.87-0.99) and the fraction of AUC extrapolated beyond the last sampling time point was less than 6%. The main pharmacokinetic parameters for flunixin are presented in Table 1.

Suppression of PG-metabolite levels

Pre-experimental levels of the PG-metabolite were high – 775 pmol/L (499-865) – in 2 goats (on 1 and 2 occasions, respectively) where the experiments were performed within 20 days after parturition (during the early postpartal period). In the remaining goats the pre-experimental levels were 66 pmol/L (43-99).

In all goats and for all routes, with one excep-

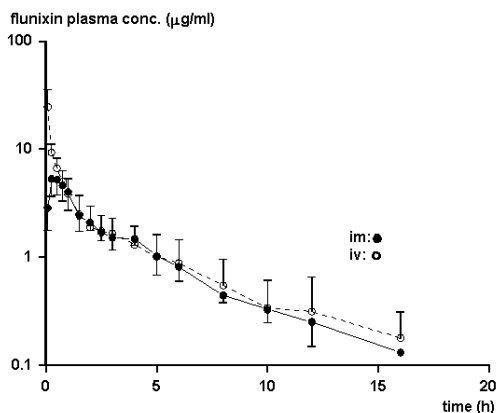


Figure 1. Mean values of the plasma concentrations obtained after intramuscular administration of flunixin to goats ($n=6$). Data after intravenous administration are shown as a reference.

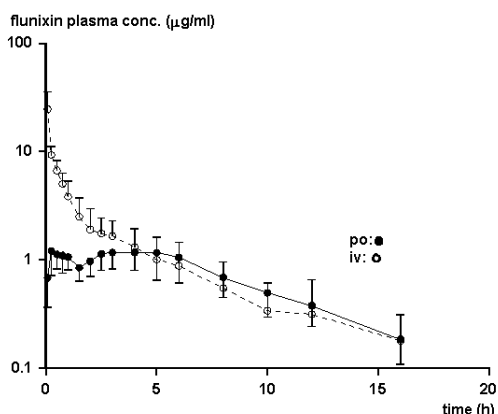


Figure 2. Mean values of the plasma concentrations obtained after oral administration of flunixin to goats ($n=6$). Data after intravenous administration are shown as a reference.

Table 1. Pharmacokinetic parameters (median (range)) in plasma following flunixin meglumine (2.2 mg/kg) given intravenously, intramuscularly and orally to 6 Norwegian dairy goats.

parameter	unit	Route of administration					
		i.v. (n=6)		i.m. (n=6)		p.o. (n=6)	
AUC	h/ μ g/ml	21	(14-36)	16	(14-19)	12	(7.5-17)
CL	ml/h/kg	110	(60-160)	-	-	-	-
Vd _{ss}	l/kg	0.35	(0.23-0.41)	-	-	-	-
λ	1/h	0.19	(0.14-0.35)	0.20	(0.10-0.27)	0.16	(0.12-0.20)
Vd _{λ}	l/kg	0.5	(0.4-0.8)	-	-	-	-
MRT	h	3.1	(2.1-5.9)	4.2	(3.5-4.5)	7.7	(6.0-9.3)
t _{1/2}	h	3.6	(2.0-5.1)	3.4	(2.6-7.1)	4.2	(3.4-6.0)
C _{max1}	μ g/ml	-	-	6.1	(3.3-7.4)	1.2	(0.8-2.0)
C _{max2}	μ g/ml	-	-	-	-	1.3	(0.8-1.8)
T _{max1}	h	-	-	0.37	(0.25-0.75)	0.37	(0.25-1.0)
T _{max2}	h	-	-	-	-	3.5	(2.5-5)
F	%	-	-	79	(53-112)	58	(35-120)

AUC = area under the concentration time curve extrapolated to infinity, CL = total body clearance, Vd_{ss} = apparent volume of distribution at steady state, λ = elimination rate constant, Vd _{λ} = volume of distribution based on the terminal phase, MRT = mean residence time, t_{1/2} = half-life of the terminal phase, C_{max} = maximum concentration, T_{max} = time for C_{max}, F = bioavailability.

tion, the plasma levels of the PG-metabolite were suppressed after administration of flunixin. In one goat, however, the concentrations of the PG-metabolite remained at approximately 60 pmol/L although assumed therapeutic exposure of flunixin (AUC = 17 h/ μ g/ml) were achieved.

The PG-metabolite levels declined 15 (5-45) min after administration of flunixin irrespectively of route of administration. The PG-metabolite levels were suppressed for a shorter time, 10 h (6-12, n=3), in goats where the experiments were performed during the early postpartal period (and the pre-experimental PG-metabolite levels were high), compared with administration later in the puerperium when the preexperimental PG-metabolite levels were low, 22 h (12-26, n=11). In 3 cases, the effect duration could not be determined, as the basal levels of the PG-metabolite were unstable (SD >20%). No statistical differences of the effect duration were found between the different routes of administration (Kruskal-Wallis, $p > 0.05$).

Discussion

In this study we have investigated the pharmacokinetics of flunixin and its ability to inhibit of prostaglandin synthesis in Norwegian dairy goats. Our main findings suggest that flunixin at a dose of 2.2 mg/kg b.w. administered either orally, intramuscularly or intravenously, suppresses the synthesis of PGF_{2 α} measured as its main metabolite, 15-ketodihydro PGF_{2 α} . Furthermore the half-life, clearance and volume of distribution at steady state in this study are comparable to reports from other ruminants like cattle (*Odensvik & Johansson* 1995), sheep (*Welsh et al.* 1993) and camels (*Wasfi et al.* 1998). Thus, the systemic exposure to flunixin after administration seem to be similar in ruminants of different species independent on route of administration.

The absorption was rapid after both intramuscular and oral administrations. After oral administration, there were two peaks in the plasma concentration during the absorption phase. This might be an effect of delayed ab-

sorption due to binding of flunixin to ruminal contents. Flunixin binds to hay and this influences the absorption in the horse (Welsh et al. 1992).

There were no statistical differences in efficacy between the different routes of administration. This is in agreement with findings in heifers (Odensvik 1995) where oral and intramuscular administration of flunixin was as efficient as intravenous administration. This suggests that the same dose could be used even though the bioavailability was only 79% and 58% after intramuscular and oral administration to goats, respectively.

Prostaglandins and thromboxanes are often used as bio-markers for COX-inhibition. As the half-lives of these compounds are very short, quantification of metabolite concentrations are used instead of the parent compounds. The use of a PG-metabolite connected to the reproductive system allowed estimation of a pharmacodynamic effect in healthy animals not subjected to inflammation or pain. This protocol had advantages for ethical reasons but had practical implications of the results. The PGF_{2α}-metabolite levels in female goats are influenced by the reproductive status and are usually elevated in the puerperium (Fredriksson et al. 1984) but within a few weeks after parturition, however, the levels decline to basal levels. The goats in our study were with 3 exceptions in the period after the puerperium and the PG-metabolite levels were basal (close to the quantification level of the assay). Due to this, it was impossible to quantify the magnitude of the PG-metabolite suppression. Instead, our interpretation of the efficacy was based on the duration rather than the magnitude. This duration was more dependent on the postpartal stage – and the pre-experimental PG-metabolite levels – than the route of administration, i.e. high PG-metabolite levels - short duration, and low levels - long duration. Similar observations have been made in

cattle where puerperal cows need flunixin administration several times per day for a more sustained inhibition of the prostaglandin synthesis (Odensvik & Fredriksson 1993).

In conclusion flunixin administered at a dose of 2.2 mg/kg orally, intramuscularly or intravenously, suppressed prostaglandin synthesis in goats. The systemic exposure of flunixin in goats was similar to that in cattle given the same dose per kg bw (which is the therapeutic dose in this species). Together these findings indicate that 2.2 mg/kg bw is likely to be an appropriate dose for clinical use also in goats.

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Sammanfattning

Farmakokinetik och farmakodynamiska effekter hos get efter intravenös, intramuskulär och oral tillförsel av flunixin.

Flunixin, dess farmakokinetik och prostaglandinsyntes-hämmande förmåga undersöktes i en studie med 6 norska mjölkgetter. Djuren gavs 2.2 mg flunixin per kg kroppsvikt och dosen tillfördes intravenöst (i.v.), intramuskulärt (i.m.) och peroralt (p.o.). Flunixinhalten i plasma analyserades med hjälp av en vätskekromatografimetod och den prostaglandinmetabolit (15-ketodihydro-PGF_{2α}) som användes för att uppskatta den prostaglandinsynteshämmande förmågan analyserades med hjälp av en radioimmunologisk metod. Resultaten presenteras som median (spridning). Halveringstiden under eliminationsfasen för de olika doseringsvägarna var: för intravenös giva.: 3,6 (2,0 - 5,0), intramuskulär giva.: 3,4 (2,6-6,8) och peroral giva.: 4,3 (3,4-6,1). Distributionsvolymen vid "steady state" var 0,35 (0,23-0,41) liter/kg och Clearance var 110 (60-160) ml/h/kg. Plasmakoncentrationen vid peroral giva uppvisade två distinkta toppar, dels vid 0,37 (0,25 - 1) timmar dels vid 3,5 (2,5 - 5) timmar efter tillförseln. Båda topparna var av samma storleksordning. Biotillgängligheten för i.m. dosering var 79% (53-112) och för peroral dosering 58% (35-120).

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