Irish Greyhound Board

Scientific Advisory Committee on Doping and Medication Control

Opinion on Carprofen

The Committee has been examining the advice it would give the Board on the threshold for carprofen in greyhound urine which could affect the performance of greyhounds.

Following an examination of the available literature, a paper was prepared on the pharmacological threshold of carprofen in plasma and urine in dogs. That paper is annexed to this opinion and formed the basis of the discussion by the Committee. The objective was to establish the levels in urine which would reflect the likely threshold in plasma/body tissues for pharmacological activity that could affect performance.

Following the discussion and further analysis the Committee agreed that the available data suggested a threshold for the racemic mixture of carprofen in urine is 10 ng/ml, and for plasma is 30 ng/ml.

Finally if further reliable date becomes available the Committee will re-examine its advice in this matter.

Carprofen – pharmacological threshold in plasma and urine in dogs

Objective

Carprofen is a non-narcotic, non-steroidal anti-inflammatory agent with characteristic analgesic and antipyretic activity. The objective of this review is to establish the threshold value for carprofen in greyhound urine which could affect the performance of greyhounds and to advise the IGB accordingly.

Chemistry

Carprofen contains an asymmetrical carbon atom and exists in two enantiomeric forms. Differences in pharmacokinetics and pharmacodynamics between the two enantiomers occur in animals and vary significantly among species. Currently, commercially available products contain a racemic (50:50) mixture of the two enantiomers, *S*(+) and *R*(−).

Pharmacodynamics

The mechanism of action of carprofen, like that of other NSAIDs, is believed to be associated with the inhibition of cyclooxygenase activity.

S(+)-enantiomer of carprofen is the anti-inflammatory part, being more than 100 times more active against COX-2 than the *R*(−)-enantiomer (ref). However, usually the potency of carprofen is expressed as the racemic mixture RS(±) carprofen. Table 1 list IC50 of Thromboxane and Prostaglandin E_2 from a number of studies which all have used the canine whole blood assay. Results show that carprofen RS(±) is more selective for COX2 than for COX1 and that IC50 of COX2 varies from 1.8 and 10 µM corresponding to 0.5 to 2.7 µg/mL.

Table 1. Inhibitory concentration (IC50) of carprofen in Canine whole blood assay.

References		COX1 (TXB2)		COX2 (PGE2)		Ratio COX1/COX2
		µg/ml	µM	µg/ml	µM	
McCann et al, 2004	RS±	18.8	68.6	2.7	10	7
Wilson et al, 2004	RS±	91		17.0		5,4
Gierse et al, 2002	RS±	32.0	117	0.5	1.8	65
Brideau et al, 2001	RS±	17.8	65	2.7	10	6,5
Lees et al, 2000	S+	48.2	176	1.9	7	25
	R-	104.0	380	44.1	161	2,4

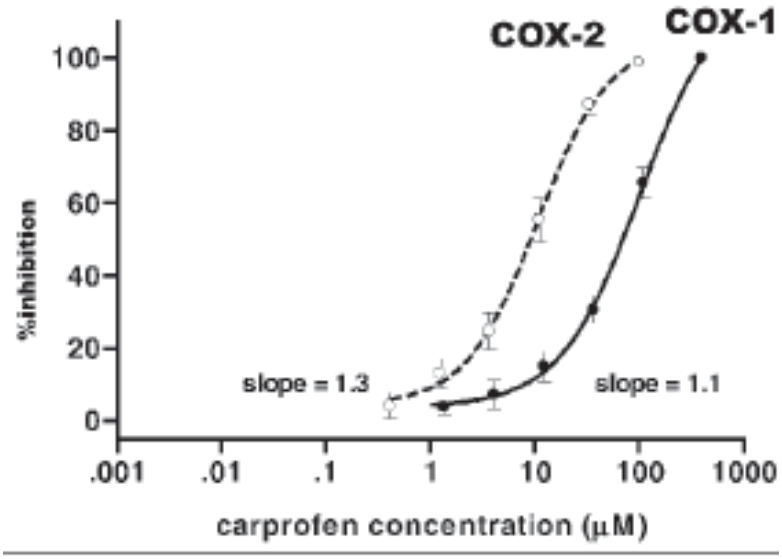

Figure 1. Concentration response curve for carprofen using Canine whole blood assay (McCain et al, 2004)

Figure 1 shows the concentration effect curve of carprofen on COX1 and COX2. At 0.1 µM (27 ng/mL) the effect of carprofen on COX2 is negligible suggesting that carprofen has no pharmacological effect at this level.

Pharmacokinetics

Fate of carprofen

Carprofen is rapidly and nearly completely absorbed (more than 90% bioavailable) when administered orally (Schmitt et al, 1990). Peak blood plasma concentrations are achieved in 1-3 hours after oral administration of 1, 5, and 25 mg/kg to dogs. The mean terminal half-life of carprofen is approximately 8 hours (range 4.5-9.8 hours) after single oral doses varying from 1-35 mg/kg of body weight (McKellar et al, 1990). After a 100 mg single intravenous bolus dose, the mean elimination half-life being approximately 11.7 hours in the dog (Schmitt M & Guentert TW, 1990). Carprofen is more than 99% bound to plasma protein and exhibits a very small volume of distribution (0.18 L/kg) (Schmitt M & Guentert TW, 1990).

Carprofen is eliminated primarily by biotransformation. Both R(−)- and S(+)-enantiomers are converted to glucuronide metabolites. Separate administration of the enantiomers shows that they are not converted to each other (McKellar et al, 1994).

In dogs 70 to 80% of carprofen metabolites are eliminated in the faeces and 10 to 20% in the urine (Rubio et al, 1980). Identified metabolites include an ester glucuronide and ether glucuronides of 2 phenolic metabolites, 7-hydroxy carprofen and 8-hydroxy carprofen. Some enterohepatic circulation of the *S*(+)-enantiomer metabolites occurs; about 34% of the dose is recirculated.

The urinary excretion of carprofen has been studied in greyhounds after an oral dose of 2.2 mg/kg bw (Dumasia et al, 2003). Carprofen, three aromatic hydroxy and a minor *N*-hydroxy metabolite were detected for up to 48 h whereas the major metabolite, α-hydroxycarprofen (coupled to glucuronic acid as an ester glucuronide) was detected for over 72 h (Table 2).

Tabel 2. Amount of carprofen (ng/mL) and peak area ratios of the major metabolite α-hydroxycarprofen in dog urine (Dumasia et al, 2003).

Urine	Time (h)	Carprofen (ng ml^{-1})		Metabolite 2 (peak area ratio)	
		Dog 4691	Dog 11775	Dog 4691	Dog 11775
U0	0	0.0	0.0	0.0	0.0
U1	2	13.0	54.6	0.37	1.7
U2	4	490.68	231.1	11.23	6.4
U3	8	358.75	195.53	12.86	8.41
U4	24	53.66	32.2	3.82	2.33
U5	48	6.85	4.9	0.57	0.43
U6	72	0.87	0.4	0.1	0.083
U7	102	0.1	0.04	0.054	0.03

Pharmacokinetic variables

Plasma concentrations of the *R*(−)-enantiomer have been reported as consistently higher than the *S*(+)-enantiomer after administration of racemic carprofen in a number of species. In dogs, one study reported the *R*(−)-enantiomer predominated in plasma (McKellar et al 1994) while another noted no significant difference in concentrations of the R and S forms (Lipscomb et al, 2002)

McKellar et al (1994) administered carprofen orally to Beagle dogs as a racemate RS(±)-CPF) at a dose of 4 mg per kg body weight and as individual (R)(-) and (S)(+) enantiomers at 2 mg per kg body weight. Each of the enantiomers achieved similar plasma bioavailability following administration as the racemate as they did following their separate administration. Only the administered enantiomers were detectable when the drug was given in the (R)(-) or (S)(+) form, indicating that chiral inversion did not occur in either direction as mentioned above.

Tabel 3. Pharmacokinetic variables of carprofen in dogs (McKellar et al, 1994)

	Treatment groups			
	RS(±)-CPF 4 mg/kg		S(+)-CPF	R(-)-CPF
	4 mg/kg		2 mg/kg	2 mg/kg
	S(+)	R(-)	S(+)	R(-)
Cmax, µg/mL	13.6 ± 0.42	17.6 ± 1.41	13.1± 3.10	12.0 ± 0.55
Tmax, h	1.0 ± 0	1.0 ± 0	0.9 ± 0.20	3.8 ± 0.75
AUC, µg/mL/h	67.1 ± 1.84	118 ± 7.64	75.2 ± 13.45	113.8 ± 9.80
Cl, mL/min/kg	0.36		0.44	0.29

Clark et al (2003) examined the steady state pharmacokinetics and bioequivalence of carprofen after oral and subcutaneous administration in beagle dogs. The racemic mixture of carprofen was administered in a dose of 25 mg pr dog (approximately 2.5 mg/kg bw) twice daily for 7 days. Plasma concentration profiles are shown in Figure 2 and pharmacokinetic variables in Table 4

Tabel 4. Pharmacokinetic variables of carprofen in beagle dogs after administration of 25 mg/dog orally or subcutaneously (Clarke et al 2003).

	Unit	Oral	Subcutaneous
Cmax	µg/mL	16.47 ± 3.95	8.08 ± 1.46
Tmax	H	1.05 ± 0.76	2.58 ± 1.64
AUC_{0-12}	µg/mL/h	71.7 ± 17.4	64.9 ± 9.7
$AUC_{0-\infty}$	µg/mL/h	89.6 ± 26.8	97.9 ± 23.4
T½	H	4.95 ± 1.32	7.07 ±2.25
Cl	mL/min/kg	0.465	0,426
F, Bioavailability	%	91,5	

Carprofen did not accumulate in the body and the pharmacokinetic variables were not different between the 1st and the 14th dose. Bioavailability after oral administration was 90% and half-life was 8.6±2.6 h.

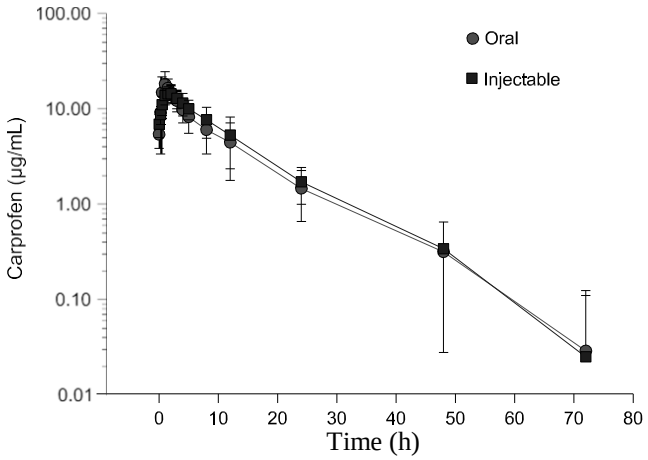

Figure 2. Carprofen plasma concentration in beagle dog after 25 mg/dog (2.5 mg/kg) orally or subcutaneously.

It is noted that pharmacokinetic variables of carprofen varies between studies. Clearance ranges from 0.28 (Schmitt M & Guentert TW, 1990), 0.36 (McKellar et al, 1994) to 0.465 ml/min/kg (Clarke et al, 2003)

Plasma threshold value for no-pharmacological effect of carprofen

Establishment of a non-pharmacological threshold value for drugs in plasma can be made in two ways: Calculating the irrelevant plasma concentration value based on effective plasma concentration of the drug combined with a safety factor or using an IC50 of a sensitive endpoint as COX2 inhibition.

Threshold value based on effective plasma concentration calculation

Effective plasma concentration (EPC) is dose*bioavailability/body clearance (per dosing interval).
Using the data from Clarke et al (2003) EPCs for RS(±)-carprofen can be calculated:
4 mg/kg*1/(0.0278 L/kg/h*24h)=2.7 mg/l (µg/ml)

Irrelevant plasma concentration (IPC) is the EPC divided by a safety factor. If the safety factor is set to 500 (50 to transform effective plasma concentration to EC50 and 10 to take individual variability into account) EPCs are:
2.7µg/ml/500=0.0053 µg/ml equivalent to 5 ng/ml

Using the clearance given by Schmitt M & Guentert TW (1990) or the clearance by McKeller et al (1994), the IPC is ending up with 15 and 20 ng/mL.

Threshold value based on COX2 inhibition

IC50 for inhibiting COX2 (PGE2) is 10 µM carprofen according to McCain et al. Considering the concentration-response curve a value of 0.1 µM (27 ng/mL) appears to give a negligible effect on COX2. Accordingly, 0.1 µM is proposed at the plasma concentration threshold. There is no need for a correction for protein binding because the assay determines the inhibiting concentration (IC) using whole blood.

Note, the threshold value in plasma derived from the two methods is at the same level.

Urinary threshold value for no-pharmacological effect of carprofen

There are no data in the literature describing the plasma urine concentration ratio for carprofen and consequently it is not possible to translate the no pharmacological threshold to a urine threshold value. Instead, the time for achieving the threshold value in plasma is estimated from the plasma concentration given by Clarke et al. (Figure 2). A plasma concentration of 27 ng/ml is achieved approximately 48 hours after a dose of 2.5 mg/kg. Translating this time point to the urinary excretion of carprofen in greyhounds give concentration of 6.85 and 4.9 ng/ml (Table 2). Since the dose in this study was 2.2 mg/kg the concentration in urine have to be corrected to the recommended dose of 4 mg/kg. This results in urinary concentrations of about 10 ng/ml.

Recommendation

Threshold values for a no-pharmacological effect of the racemic mixture of carprofen are:

Plasma: 30 ng/mL

Urine: 10 ng/mL

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