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Effect of an endurance-like exercise on the disposition and detection time of phenylbutazone and dexamethasone in the horse: Application to medication control

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Keywords: horse; doping; detection time; dexamethasone; phenylbutazone; exercise

Summary

Reasons for performing study: **Equine antidoping rules were established to prevent a horse's performance being altered after the administration of prohibited substances, including approved drugs used for legitimate treatment. Veterinarians have to advise owners or trainers on appropriate withholding times to guarantee that their horses may safely compete after drug administration. In order to propose tailored withdrawal times, several horse organisations released detection time (DT) values, for the main veterinary drugs used in horses. One of the possible limits to the information provided by published DTs in horses is the fact that they are determined from classic pharmacokinetic studies performed at rest under laboratory conditions. In field conditions, training and exercise programmes may have an influence on drug elimination.**

Methods: **Dexamethasone (DMX) and phenylbutazone (PBZ) have been quantified in plasma and urine after solid phase extraction. The kinetic disposition of DXM (8 µg/kg) and PBZ (8 mg/kg) administered by i.v. route in 8 horses, was investigated in rest conditions and during a standardised 3 h test exercise according to a cross-over design.**

Objectives: **The aim of the present study was to compare the kinetic disposition of 2 test drugs, DMX and PBZ in rest vs. exercising conditions.**

Results: **It was shown in 8 horses that a sustained 3 h of mild exercise slightly decreased the plasma clearance of both drugs (about 25% for DXM and 37% for PBZ) and this is mainly explained by the significant decrease of the corresponding hepatic clearance. In addition, as the volume of distribution was correlatively decreased, the plasma terminal half-life, which is a hybrid parameter of plasma clearance and volume of distribution, remains unchanged overall.**

Conclusion and potential relevance: **Establishing DTs or withdrawal times (WTs) are relevant as plasma and urine half-lives, but not clearance, are the main determinants of DT length. Veterinarians may realistically decide upon a WT for a legitimate drug based on the corresponding DT obtained under resting conditions providing this drug has a low hepatic extraction ratio and a safety margin is added to allow for all possible sources of variability.**

Abbreviations

DT:	Detection time
EHSLC:	European Horseracing Scientific Liaison Committee
FEI:	Fédération Equestre Internationale
HSL:	Harmonized Screening Limit
WT:	Withdrawal time

Introduction

The horse racing industry and equestrian sport acknowledges that legitimate drug treatment must be applied when necessary and makes a clear distinction between medication control (legitimate drug) and 'doping' control (illegal substances). To detect drug exposure, sophisticated analytical techniques are used and any trace of a prohibited substance often constitutes a 'doping' offence. Although appropriate for 'doping' control, such an approach, known as the 'zero tolerance rule' is not suitable for medication control (Smith 2000) because very sensitive analytical detection methods mean irrelevant amounts of therapeutic substances may now be detected a long time after their administration. As a solution to this dilemma, the European Horseracing Scientific Liaison Committee (EHSLC) has recommended to European racing laboratories involved in its programme to apply an Harmonized Screening Limit (HSL), which appropriately limits the sensitivity of analytical techniques used for medication control (Barragry 2006).

However, as these HSLs are not made public, they are of no practical value for veterinarians who must advise owners or trainers on appropriate withholding times to guarantee that their horses may safely compete after drug administration. In order to help veterinarians to propose tailored withdrawal times, the EHSLC decided to determine corresponding detection times, for the main veterinary drugs used in horses. A detection time (DT), according to the EHSLC definition, is the time at which the urinary (or plasma) concentrations of a drug, in all horses involved in a particular trial, are observed to be

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lower than the HSL when controls are performed using routine screening methods. It should be stressed that the DTs, as issued by the EHSLC (and followed by FEI), are not synonymous with withdrawal times (WT). A DT is a raw experimental observation, whereas a WT is a recommendation and, as such, is a matter for the professional judgement of the treating veterinarian. A WT should be longer than a DT because the WT should take into account the impact of all sources of animal variability (e.g. age, sex, breed, training, racing, etc.) and those of the medicinal product actually administered (e.g. formulation, route of administration, dosage regimen, duration of treatment, etc.) in order to avoid a positive control.

One of the possible limits to the information provided by published DTs in horses is the fact that they are determined from classic pharmacokinetic studies performed at rest under laboratory conditions. In field conditions, training and exercise programmes may have an influence on drug elimination. In horses, there is practically no experimental information on the direct effect of exercise on drug disposition. Prolonged submaximal exercise has not been shown to influence plasma clearance of frusemide measured several minutes after the end of the test exercise (Dyke *et al.* 1996). On the other hand, several studies have described the effects of exercise on functions involved in drug disposition (Poortmans 1977; Ylitalo 1991; McKeever *et al.* 1993; Manohar *et al.* 1995). In horses, hepatic blood flow is significantly decreased during submaximal exercise and it was hypothesised that drugs that are efficiently extracted by the liver may have decreased hepatic clearance when horses are subjected to submaximal exercise (Dyke *et al.* 1998a). For the kidney, it was shown that the glomerular filtration rate fell over 40% during mild exercise in humans (Gleadhill *et al.* 2000). As the kidney and liver are the 2 main organs involved in drug elimination, it is reasonable to suppose that the cardiovascular adaptation observed during sustained exercise can lead to changes in drug disposition and consequently, to a different DT from that obtained at rest.

To explore the possible influence of exercise on the DT as obtained under the EHSLC conditions, the aim of the present study was to compare the kinetic disposition of 2 test drugs in rest conditions and in conditions involving a 3 h endurance-type exercise. As it has been reported that exercise decreases the distribution and excretion of flow-limited drugs and increases the distribution and excretion of capacity-limited drugs (Somani *et al.* 1990), dexamethasone, a drug having a rather high clearance rate and phenylbutazone, a drug with a low clearance rate, were selected as test drugs.

Materials and methods

Animals

Eight healthy, mature sport horses (6 geldings, 2 females; age 5–17 years; weighing 430–618 kg), participating on a regular basis in equestrian competitions, were selected initially in this study. The horses were randomly placed into 2 groups of 4. The same horses were used for the testing of both drugs such that the groups remained the same for the entire study; except for one gelding that participated in the dexamethasone (DXM) trial but had to be replaced by a new subject for the phenylbutazone (PBZ) trial because it was too difficult to sample during exercise conditions. Horses were housed in individual boxes and/or paddocks for the duration of the study. Feed ration was identical for all horses and included complete pelleted diet as well as hay. Straw bedding was used and water was available *ad libitum*.

Experimental design

The kinetic dispositions of DXM and PBZ were studied in 2 different trials and following an identical 2-period cross-over design for each of the 2 tested drugs. One group of horses was first tested under rest conditions and then under exercise conditions and *vice versa* for the second group. For each trial, there was a washout period of 4 weeks between the 2 phases and there was also a washout period of 4–5 weeks between the 2 trials.

Exercise protocols

All exercise tests were conducted at the Cépière racetrack in Toulouse, France on an oval sand track of approximately 1100 m in length. The standardised training sessions performed 2 weeks prior to each exercise phase of the study were designed to represent approximately half the pivotal endurance test exercise. The 3 h test exercise included walking, trotting and canter sequences as described in Figure 1. During the exercise phases, the average speed was approximately 12 km/h. After the first 90 mins, the horses were returned to their stalls and offered water *ad libitum* during approximately 30 mins before returning for the second half of the test exercise. In order to ascertain that the horses were all performing at a standardised level over the entire exercise period, individual heart rates were recorded using heart rate monitors (Polar Equine S625X monitors)[1].

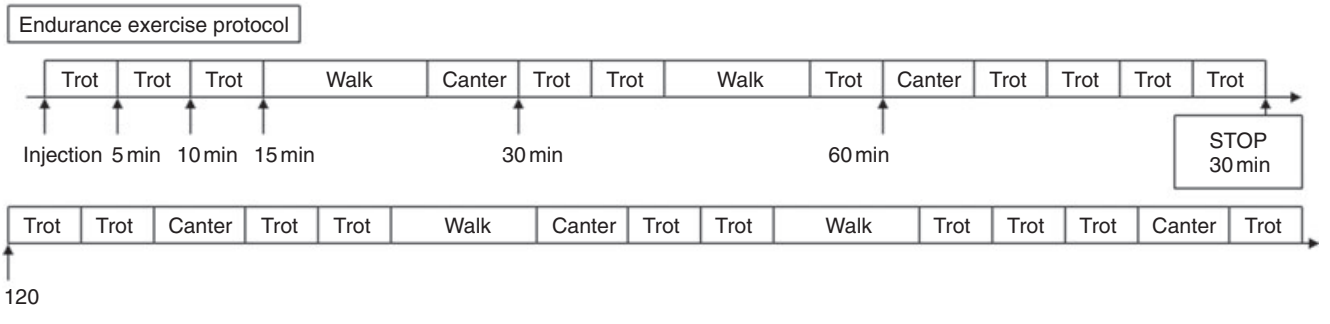

Fig 1: Schematic representation of the endurance exercise protocol used in this study for both dexamethasone and phenylbutazone exercise conditions.

Drug administration

Horses were weighed no more than 7 days prior to the start of each study phase. An indwelling i.v. catheter was placed in the right jugular vein of each horse just prior to drug administration either on the racetrack (trial during exercise) or in an individual stall (trial at rest). DXM or PBZ were then administered i.v. at 8 µg/kg or 8 mg/kg, respectively, and the catheter removed immediately.

Drugs and chemicals

DXM alcohol (Cortamethasone) and PBZ (Phenyarthite) were obtained from Vetoquinol[2].

Blood sampling

All blood samples for the determination of plasma drug concentrations were collected from the left jugular vein. For each condition (rest or exercise) and for both drugs, a blood sample was collected just prior to drug administration, at 5, 10, 15, 30 and 60 mins, and at 2, 4, 6, 8, 24, 32 and 48 h post injection. For blood collection during the exercise phase, horses were stopped no more than 3 mins for each sampling time. Thirty ml blood samples were obtained using heparin vacutainer tubes (BD Vacutainer[3], LH 170 iu ref 367526) and centrifuged at 4000 ***g*** for 10 mins within 30 mins of collection. Three aliquots of each plasma sample were then stored on ice and subsequently frozen at -20°C until analysis.

Urine sampling

A single 15 ml urine sample was collected before drug administration. *Ad hoc* designed urine collection halters were used to collect total urine by natural micturition for 4 h following drug administration as well as from 24–28 h after drug administration for another 4 h collection period. During these collection periods, total urine volume and pH were recorded for each individual micturition. At the end of each 4 h collection period, all urine samples collected were pooled and three 15 ml aliquots were kept frozen at -20°C until analysis. Single 15 ml urine samples were also collected on days 7, 8 or 9 for both drugs, on Days 15, 16, 17, 21, 22 or 23 for DXM and on Days 24 or 25 for PBZ. Aliquots of these samples were also kept frozen at -20°C until analysis. Urine pH was measured with a pH meter (Mettler Toledo MP230, Viroflay, France)[4] after each micturition during the total urine collection periods.

Sample analysis

DXM concentrations were quantified in plasma and urine by HPLC/ESI-MS positive mode on a Quantum Ultra apparatus (Thermo Scientific[5]) after extraction on Varian C18HF cartridges[6] using flumethasone as an internal standard (IS). DXM was quantified in SRM mode with mean of the selected fragment ions at m/z 361 and m/z 379 for IS.

Detection limits for DXM were 10 pg/ml in plasma and 5 pg/ml in urine with a quantification limit of 25 pg/ml for both media. For plasma and urine, linearity was observed from 25 pg/ml to 5000 pg/ml. In plasma, the precision and accuracy were determined at 250,750 and 3000 pg/ml and in urine at 50,500 and 3000 pg/ml. The accuracy ranged 104–111% and the within-day and day-to-day precisions were all less than 10%.

PBZ in plasma and PBZ at low concentrations in urine were quantified by HPLC/ESI-MS in positive ion mode on a Trap XCT Plus apparatus (Agilent Technologies[7]). PBZ at high concentrations in urine was quantified by GC/EI-MS on an HP MSD 5973 apparatus (Agilent Technologies) after extraction on OASIS HLB 3 cc (60 mg) cartridges with tolfenamic acid as IS. Extraction was conducted on Varian Nexus HFC18 cartridges (3 cc/500 mg) with phenylpropazone (PPZ) as IS in urine and tolfenamic acid in plasma. In the SRM mode, the fragment ions used for quantification were ions at m/z 279 for PBZ, m/z 216 for tolfenamic acid and m/z 265 for PPZ. In GC/MS, fragment ions selected for quantification were m/z 77, m/z 183, and m/z 308 for PBZ and m/z 294 and m/z 77 for PPZ.

The validation parameters for PBZ quantification in plasma were linearity from 20 ng/ml to 200 ng/ml (a weighting factor of $1/X^2$ was applied) and within-day and day to day precisions for PBZ concentrations at 50 and 150 ng/ml of <10% (accuracy was 93% at both concentrations). The limit of detection was 1 ng/ml and the quantification limit was 5 ng/ml. In urine, by GC/MS, linearity was observed from 25 ng/ml to 100 ng/ml using a weighting factor of $1/X^2$. Within-day and day-to-day precisions for PBZ concentrations at 50, 250 and 500 ng/ml were less than 15% (accuracy ranged 104–109%).

Low concentrations of PBZ in urine were quantified by LC/MS. Linearity was observed from 2.5 ng/ml to 100 ng/ml using a weighting factor of $1/X^2$. Within-day and day-to-day precisions for PBZ concentrations at 10 and 75 ng/ml were less than 15% (accuracy was 94 and 96%, respectively). The limit of detection was 1 ng/ml and the quantification limit was 5 ng/ml.

Pharmacokinetics analysis

A noncompartmental pharmacokinetic analysis (NCA) was performed using WinNonlin Professional software (WinNonlin, Version 5.2)[8].

Plasma clearance (Cl) was obtained using *equation 1*:

$$Cl_{tot} = \frac{Dose}{AUC} \qquad \textit{Equation 1}$$

Where Dose is the administered DXM or PBZ dose and AUC is the total area under the plasma concentration vs. time curve calculated for each horse by use of the linear trapezoidal rule with extrapolation to infinity.

The mean residence time (MRT), steady-state volume of distribution (V_{ss}) and the terminal half-life were computed in accordance with classical equations (Gibaldi and Perrier 1982)

Renal clearances for DXM and PBZ were calculated using *equation 2*:

$$Cl_r = \frac{Amount\_of\_drug\_in\_urine_{over\_4h}}{AUC_{over\_4h}} \qquad \textit{Equation 2}$$

Where *Amount of drug in urine* is the total amount of DXM or PBZ eliminated in urine over a collection time interval of 4 h and *AUC* is the corresponding plasma DXM or PBZ AUC (i.e. $AUC_{(0\text{-}4h)}$ and $AUC_{(24\text{-}28h)}$) obtained using the linear trapezoidal rule and the NCA module of WinNonlin. Urine was collected on 2 occasions: during the first 4 h following drug administration (i.e. during the exercise period itself for the test exercise phases of the study) and from 24–28 h after drug administration.

Hepatic DXM or PBZ clearance (Cl_h) was estimated by *equation 3*:

$$Cl_h = Cl_{tot} - Cl_r \qquad \textit{Equation 3}$$

where Cl_{tot} is the plasma clearance as obtained using *equation 1* and Cl_r the renal clearance as obtained using *equation 2*. It was assumed that there was no other relevant source of drug elimination other than kidney and liver for both drugs (see *Discussion*).

The hepatic extraction ratio was estimated from the ratio of the hepatic plasma clearance over the hepatic blood flow fixed at 20 ml/kg bwt/min for either rest or exercise, as proposed by (Dyke *et al.* 1998a).

Statistical analysis

Descriptive values were reported as mean ± s.d. Statistical analysis was performed by use of a statistical program (SAS release 9.1)[9]. For each plasma pharmacokinetic parameter of interest as well as for urine pH, a repeated-measures linear model with status (exercise vs. rest) determined as an intrasubject factor and period (1 or 2) as an intersubject factor, was used to determine the effect of exercise on the disposition of each drug.

A significance level of $P<0.05$ was used for the interpretation of all statistical analyses.

Results

The mean heart rates for a given gait were not significantly different between subjects, between periods for a given drug or between drugs during exercise ($P>0.05$). This indicated an appropriate standardisation of the test exercise and an a priori comparability of horses.

Figures 2 and 3 show the plasma concentrations (mean ± s.d.) vs. time curves at rest vs. during exercise up to 48 h post drug administration for DXM and PBZ, respectively. Mean plasma concentrations vs. time curves for the first 4 h following drug administration are shown for DXM and PBZ in Figures 4 and 5, respectively. Individual pharmacokinetic parameters are given in Table 1 for DXM and Table 2 for PBZ. The urine pH measured in the pooled urine obtained over the first 4 h following drug administration was similar for both drugs and both conditions (rest vs. exercise); the mean values ranged 8.1–8.4.

For all horses, urinary concentrations of DXM were undetectable by Day 7 (LOD = 5 pg/ml). For PBZ at Day 7, the mean (± s.d.) PBZ concentrations were 42.5 ± 20.6 and 36.8 ± 20.3 ng/ml for rest and exercise conditions, respectively. Only 2 horses had detectable PBZ levels on Day 24 (LOD = 1 ng/ml).

When drugs were administered just before the 3 h test exercise, the plasma clearance was decreased for both DXM (-23.9%) and PBZ (-36.8%) but the difference was only significant for DXM ($P = 0.034$) and not for PBZ ($P = 0.084$). Similarly, the volume of distribution (V_{ss}) decreased with exercise for both DXM (-17.2%) and PBZ (-41.7%) but the difference was only significant for DXM ($P = 0.0035$) and not for PBZ ($P = 0.19$).

For DXM, the renal clearance at rest represented only, on average, 5.2% of the total plasma clearance, suggesting that hepatic clearance is the main pathway of DXM elimination in the horse. Under test exercise conditions, the contribution of renal clearance to DXM elimination was very similar (5.4%) to that measured under resting conditions ($P>0.05$). For DXM, the hepatic clearance was significantly decreased when DXM was administered just before the test exercise (-22.8%, $P = 0.02$). For PBZ, the contribution of renal clearance was very similar at rest and under test exercise conditions (11.2 and 12.9%, respectively, $P>0.05$). Hepatic clearance was the most important pathway for PBZ elimination and was significantly decreased (-25.3%, $P = 0.02$) in test exercise conditions.

For both drugs, the terminal half-life remained unchanged during both conditions ($P>0.05$); it was about 4–4.5 h for DXM and 7.4 h for PBZ.

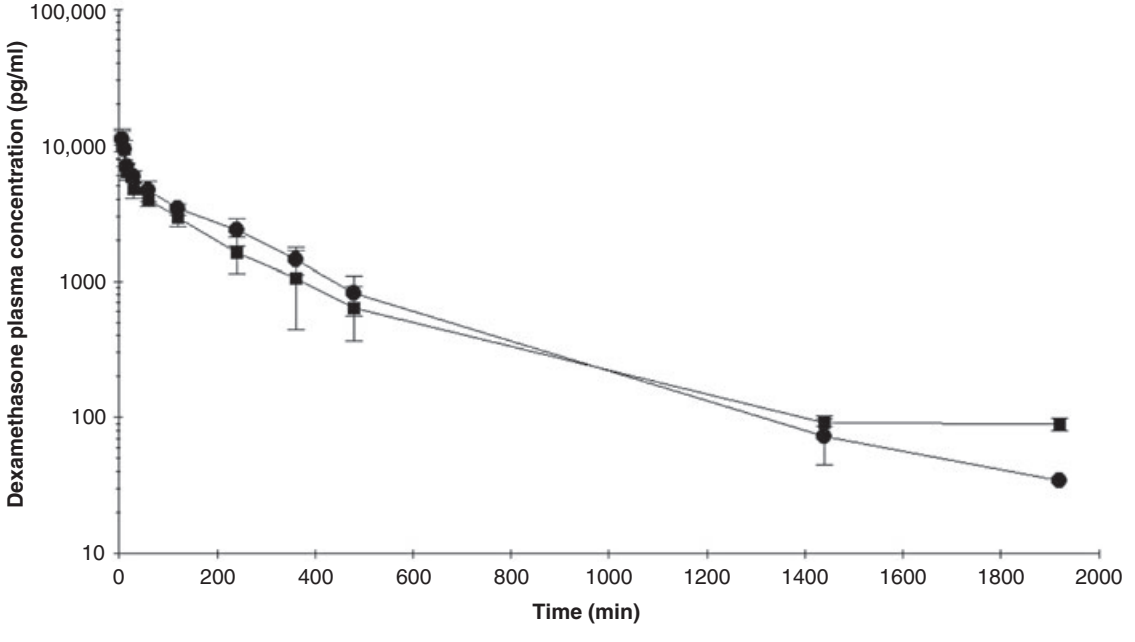

Fig 2: Semilogarithmic plot of the mean (± s.d.) plasma concentrations of dexamethasone in 8 horses after i.v. dexamethasone administration (8 µg/kg bwt) administered during rest conditions (■) or just before a 3 h standard test exercise (●).

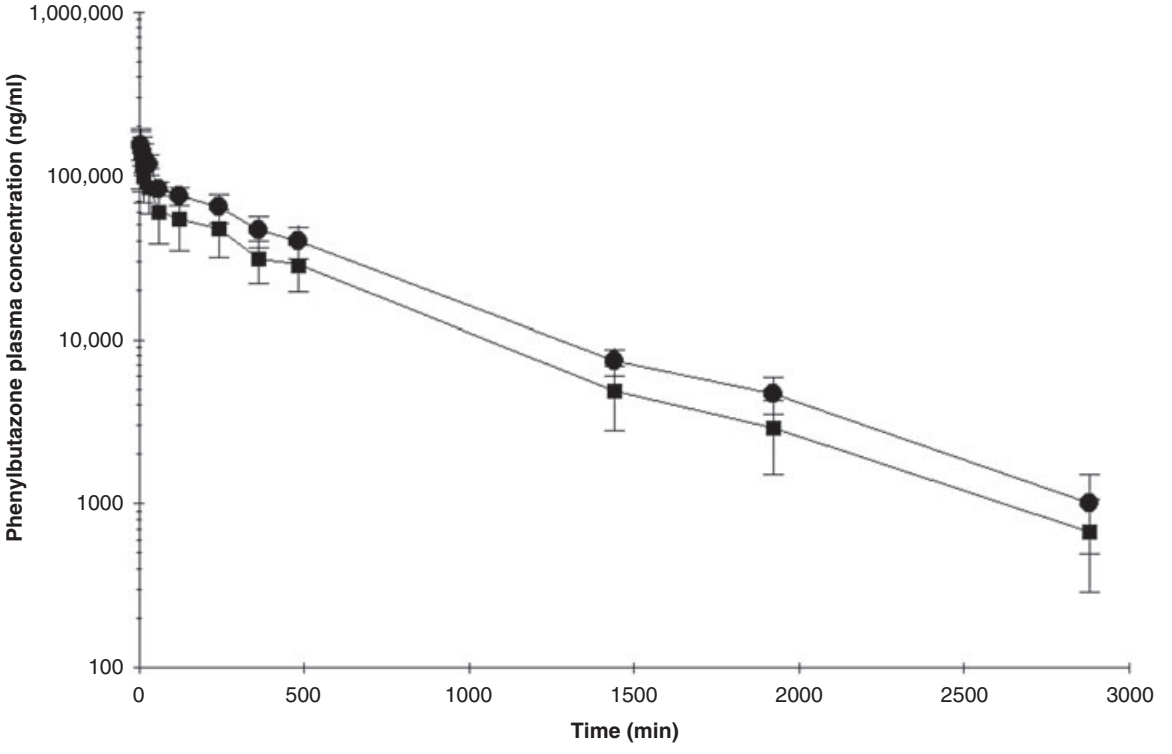

Fig 3: Semilogarithmic plot of the mean (± s.d.) plasma concentrations of phenylbutazone in 8 horses after i.v. phenylbutazone administration (8 mg/kg bwt) administered during rest conditions (■) or just before a 3 h standard test exercise (●).

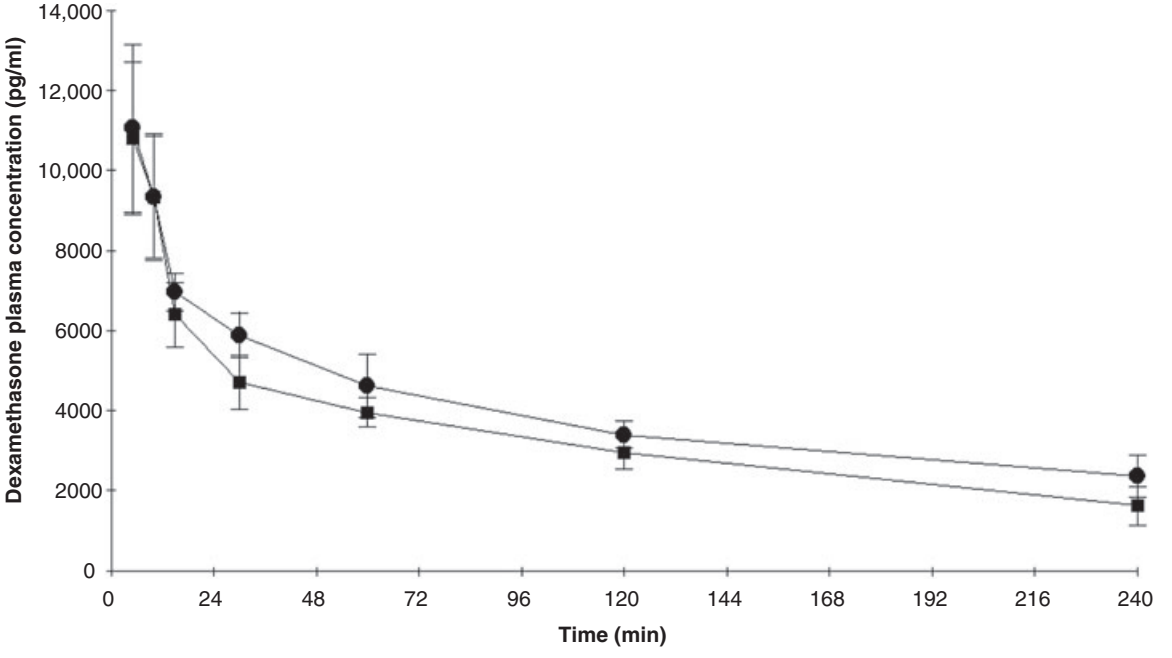

Fig 4: Arithmetic plot of the mean (± s.d.) plasma concentrations of dexamethasone in 8 horses for the first 4 h following i.v. dexamethasone administration (8 μg/kg bwt) administered during rest conditions (■) or just before a 3 h standard test exercise (●).

Discussion

A sustained mild test exercise slightly decreased the plasma clearance of both investigated drugs (about 25% for DXM and 37% for PBZ) and this decrease can be explained by the significant decrease of the corresponding hepatic clearance. In addition, as the volume of distribution was correlatively decreased, the plasma terminal half-life, which is a hybrid parameter of plasma clearance and volume of distribution, remained unchanged overall. This is of relevance for establishing detection times (DTs) or withdrawal times (WTs) as plasma and urine half-lives, but not clearance, are the main determinants of DT (Toutain 2010).

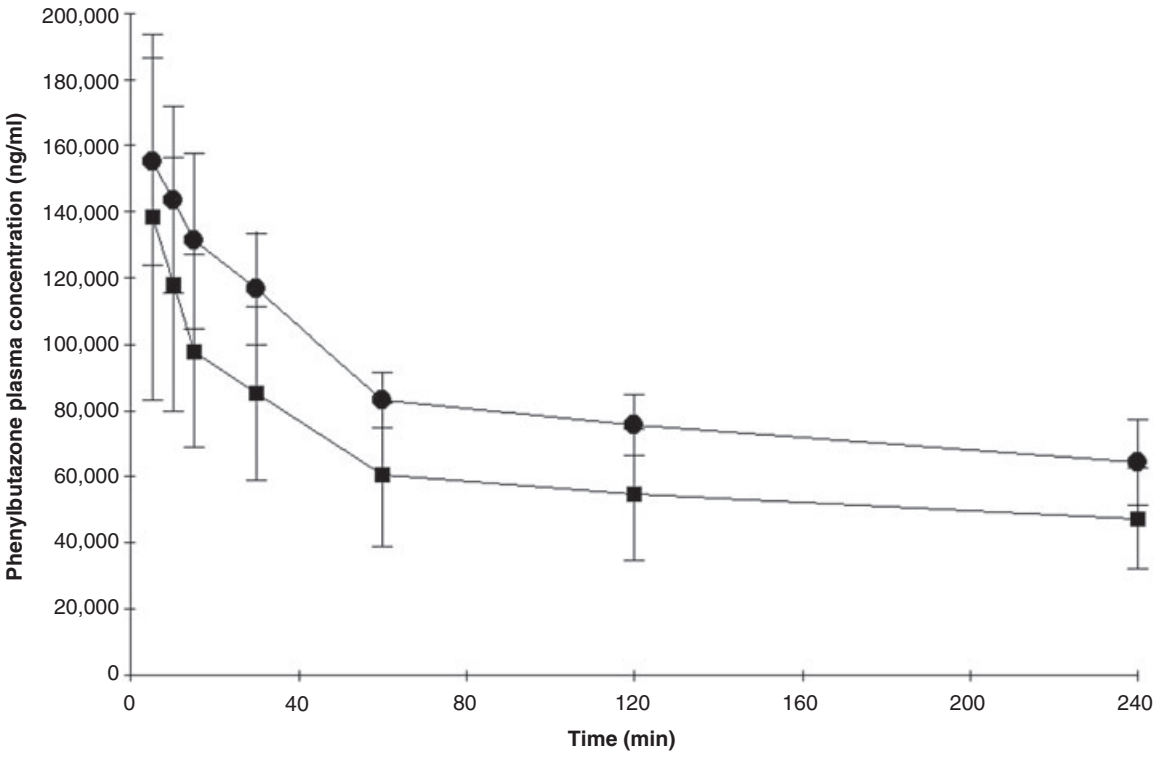

Fig 5: Arithmetic plot of the mean (± s.d.) plasma concentrations of phenylbutazone in 8 horses for the first 4 h following i.v. phenylbutazone administration (8 mg/kg bwt) administered during rest conditions (■) or just before a 3 h standard test exercise (●).

TABLE 1: Pharmacokinetic parameters of dexamethasone administered by i.v. route (8 μg/kg bwt) to 8 horses at rest vs. the same horses just before a 3 h standardised test exercise (mean ± s.d.)

	Plasma half-life (h)	AUC_{tot} (μg bwt/h/l)	Cl_{tot} (ml/kg bwt/h)	MRT (h)	V_{ss} (l/kg)	Cl_r (ml/kg bwt/h)	Cl_h* (ml/kg bwt/h)	Cl_{r_ratio} (%)
Rest	4.54 ± 0.57	23.92 ± 5.81	357 ± 110	4.38 ± 1.31	1.45 ± 0.79	16.53 ± 16.35	325	5.2 ± 5.1
Exercise	4.01 ± 0.57	29.90 ± 4.17	272 ± 37	4.47 ± 0.44	1.20 ± 0.25	14.87 ± 5.11	257	5.4 ± 2
P value	>0.05	0.003	0.04	>0.05	0.004	>0.05	0.02	>0.05

*Average Cl_h was estimated by the least square mean from the general linear model; P values comparing exercise vs. rest were obtained from the general linear model. AUC_{tot} = area under plasma concentration vs. time curve; Cl_{tot} = plasma clearance; MRT = mean residence time; V_{ss} = steady-state volume of distribution; Cl_h = hepatic clearance; Cl_{r_ratio} = ratio of renal clearance to plasmatic clearance.

TABLE 2: Pharmacokinetic parameters of phenylbutazone administered by i.v. route (8 mg/kg) in 8 horses at rest vs. in the same horses just before a 3 h standardised test exercise (mean ± s.d.)

	Plasma half-life (h)	AUC_{tot} (μg bwt/h/l)	Cl_{tot} (ml/kg bwt/h)	MRT (h)	V_{ss} (l/kg bwt)	Cl_r (ml/kg bwt/h)	Cl_h* (ml/kg bwt/h)	Cl_{r_ratio} (%)
Rest	7.42 ± 1.61	723± 215	12.66 ± 6.58	8.89 ± 1.82	0.12 ± 0.11	1.17 ± 0.65	9.18	11.2
Exercise	7.43 ± 0.99	1021 ± 153	7.99 ± 1.13	9.03 ± 0.84	0.07 ± 0.01	0.99 ± 0.41	6.87	12.9
P value	>0.05	0.02	>0.05	>0.05	>0.05	>0.05	0.02	>0.05

*Average Cl_h was estimated by the least square mean from the general linear model; P values comparing exercise vs. rest were obtained from the general linear model. AUC_{tot} = area under plasma concentration vs. time curve; Cl_{tot} = plasma clearance; MRT = mean residence time; V_{ss} = steady-state volume of distribution; Cl_h = hepatic clearance; Cl_{r_ratio} = ratio of renal clearance to plasmatic clearance.

This study tested the influence of exercise on the kinetic disposition of 2 representative drugs and assessed a possible alteration of DTs by exercise. Currently, all investigations conducted by the EHSLC, the results of which are shared with the FEI, are carried out under resting conditions while most of the medication controls relate to post racing conditions. An endurance-like exercise was chosen for this study because it was assumed that this type of exercise represented a worst case scenario: most race horses expend energy for a much shorter duration. A similar reduction in the plasma clearance of sulfobromophthalein, when horses were subjected to submaximal exercise intensities reaching 40, 60 and 80% of maximal oxygen consumption, has been shown (Dyke *et al.* 1998a,b). This suggests that the intensity of the present test exercise (estimated to be about 40–50% of VO_2max) was

sufficient to alter both the hepatic blood flow (Dyke *et al.* 1998a) and the kidney function (Gleadhill *et al.* 2000).

In the present experiment, both renal and hepatic clearances were estimated for PBZ and DXM in an attempt to explain the origin of a possible alteration of the total plasma clearance, which controls overall drug exposure. A significant decrease in hepatic clearance was shown for both drugs with no evidence of an alteration in the corresponding renal clearance. Hence, for both drugs, the hepatic clearance was the main contributor to the total plasma clearance (about 95 and 90% for DXM and PBZ, respectively). A hepatic clearance at rest for PBZ of 0.16 ml/kg bwt/min should be considered as very low given that the hepatic blood flow in horses is about 20 ml/kg bwt/min (Dyke *et al.* 1998a) and represents an estimated hepatic extraction ratio of PBZ of less than 1%. For DXM, the hepatic clearance was 5.47 ml/kg bwt/min leading to an estimated hepatic extraction ratio of about 27%, which is considered as the upper limit for low hepatic extraction drugs (Rowland and Tozer 1995).

When hepatic clearance is low (0.16 ml/kg bwt/min for PBZ) or rather low (5.47 ml/kg bwt/min for DXM), it can be modelled by the following classic *equation 4*:

$$Cl_h = fu \times Cl_{\text{int}} \qquad \textit{Equation 4}$$

Where *fu* (from 0 to 1) is the free drug fraction and Cl_{int}, the intrinsic clearance that reflects drug metabolism. In the experimental conditions of this study, it is possible that the observed reduction of hepatic clearance was mainly due to a decrease in *fu* that could be related to an increase in the plasma protein binding of PBZ and DXM. This hypothesis is consistent with the fact that the volume of distribution (V_{SS}) was also reduced. Such a reduction may have several origins, including an increase in plasma albumin. Previously, it was observed that a standardised exercise test (equivalent to 50% of the exercise intensity used in this study) in horses resulted in a significant average increase in plasma albumin concentration: 14% after only 20 mins of effort (Graham-Thiers *et al.* 2003).

The hypothesis of a reduction in *fu* is consistent with the fact that the plasma half-life of DXM and PBZ remained unchanged under test exercise conditions. Indeed, the plasma terminal half-life is a hybrid pharmacokinetic parameter dependant on the ratio of volume of distribution and plasma clearance (Toutain and Bousquet-Melou 2004).

The main motivation of the present study was to assess the possible influence of exercise on the DT of 2 representative drugs. Accepting that the plasma or urine terminal half-lives are the main determinants of a DT rather than plasma clearance (Toutain 2010), and that for both drugs the terminal half-life was unchanged by exercise, it can be suggested that the DT of both PBZ and DXM are likely to be the same in resting and exercise conditions. This conclusion is supported by the direct measurement of PBZ in urine at the time corresponding to the DT given by the EHSLC and FEI (7 days); the mean urine PBZ concentrations were seen to be very similar for both conditions with an overall average value of 37 ng/ml.

The results obtained of this work are similar to those obtained by others for PBZ (Soma *et al.* 1983) and DXM (Cunningham *et al.* 1996). For these 2 drugs, at least, which have low hepatic extraction ratios, the results provide evidence that kinetic parameters, obtained at rest for the determination of DTs, are relevant for medication control in post racing conditions. More generally, it can be hypothesised that a race lasting only a few minutes will not dramatically alter residual drug concentrations in plasma or urine at the control sampling times, i.e. at a time when most of the drug has been already eliminated.

In conclusion, this study suggests that veterinarians may reasonably decide upon a WT for a legitimate drug based on the corresponding DT obtained under resting conditions, providing the drug in question has a low hepatic extraction ratio and a safety margin is added to allow for all possible sources of variability.

Acknowledgements

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Manufacturers' addresses

[1]Polar Company, Kempele Finland.
[2]Vetoquinol, Lure, France.
[3]Beckman Dickinson, Plymouth, UK.
[4]Mettler Toledo, Viroflay, France.
[5]Thermo Scientific, Courtaboeuf, France.
[6]Varian, Les Ulis, France.
[7]Agilent Technologies, Palaiseau, France.
[8]Pharsight Corporation, Mountain View, California, USA.
[9]SAS Institute Inc., Cary, North Carolina, USA.

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