

# Pharmacokinetics of intra-articular betamethasone sodium phosphate and betamethasone acetate and endogenous hydrocortisone suppression in exercising horses

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Menendez, M.I., Phelps, M.A., Bertone, A.L. Pharmacokinetics of intra-articular betamethasone sodium phosphate and betamethasone acetate and endogenous hydrocortisone suppression in exercising horses. *J. vet. Pharmacol. Therap.* doi: 10.1111/jvp.12229.

To the date, no reports exist of the pharmacokinetics (PK) of betamethasone (BTM) sodium phosphate and betamethasone acetate administered intra-articular (IA) into multiple joints in exercising horses. The purpose of the study was to determine the PK of BTM and HYD concentrations in plasma and urine after IA administration of a total of 30 mg BTM. Eight 4 years old Thoroughbred mares were exercised on a treadmill and BTM was administered IA. Plasma and urine BTM and HYD were determined via high performance liquid chromatography spectrometry for 6 weeks. Concentration-time profiles of BTM and HYD in plasma and urine were used to generate PK estimates for non-compartmental analyses and comparisons among times and HYD concentrations. BTM in plasma had greater  $T_{max}$  ( $T_{max}$  0.8 h) vs. urine ( $T_{max}$  7.1 h). Urine BTM concentration (ng/mL) and amount ( $AUC_{last}$ ; h  $\times$  ng/mL) were greater than plasma. HYD was suppressed for at least 3 days ( $<1$  ng/mL) for all horses. The time of last quantifiable concentration of BTM ( $T_{last}$ ; hour) was not significantly different in plasma than urine. Use of highly sensitive HPLC-MS/MS assays enabled early detection and prolonged and consistent determination of BTM in plasma and urine.

(Paper received 3 December 2014; accepted for publication 12 March 2015)

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## INTRODUCTION

Intra-articular (IA) medications are a routine part of management of horses in training and to assist with reducing joint inflammation and pain. Glucocorticoids (GCs) are the most potent and frequently used IA medication with respect to anti-inflammatory activity and have served as a pillar in joint treatment (Autefage *et al.*, 1986; Toutain *et al.*, 1988; Soma *et al.*, 2006, 2011; Menendez *et al.*, 2012). The level of competition and importance of safety of the equine athletes together with the public scrutiny on our competitive horses justifies pertinent withdrawal times of IA GCs to establish blood concentrations below permissible limits. The United States Equestrian Federation (USEF) regulates detection times and establishes Screening Limits of Detection (SLOD) for drugs in equine competition, providing withdrawal guidelines for controlled therapeutic medications. The Federation Equestre Internationale (FEI) publishes a list of prohibited substances and their detection times. The Racing Medication & Testing Consortium (RMTC) recommends drug

thresholds in the USA ([www.rmtcnet.com](http://www.rmtcnet.com)). To assure a clear distinction between the use of routine, legitimate medication and deliberate and calculated doping to affect a horse's performance, USEF published a Drugs & Medications guideline document (2014 USEF Rule Book; [www.usef.org](http://www.usef.org)).

Celestone Soluspan<sup>®</sup> (betamethasone sodium phosphate and betamethasone acetate (BTM)) is an aqueous suspension containing water-soluble betamethasone (sodium phosphate) and water-insoluble betamethasone (acetate) for intramuscular (i.m.) or IA administration. This drug is approved for human use, and the drug is still not approved for veterinary use in the U.S. The sodium phosphate provides the GC short action, and the acetate formulation is repository, delays systemic absorption and prolongs the duration of action (Ferguson & Hoenig, 2001). The hypothalamus–pituitary–adrenal axis regulates the endogenous secretion of GCs. Endogenous and exogenous GCs have a regulatory effect on the hypothalamus–pituitary–adrenal axis. (Autefage *et al.*, 1986; Toutain *et al.*, 1988; Soma *et al.*, 2006, 2011; Menendez *et al.*, 2012). Plasma

hydrocortisone (HYD) has served as an indicator of systemic endogenous cortisone production and may be suppressed by even low level of BTM administration. The absence of HYD in plasma may suggest administration of exogenous GCs. Decreased plasma concentrations of endogenous GCs following IA administration of methylprednisolone acetate (MP) in horses have been detected for 264 h (Soma *et al.*, 2006). Our study in exercised horses showed HYD decreased for 18 and 39 h after IA dose of 100 and 200 mg of MP, respectively (Menendez *et al.*, 2012).

The level and duration of cortisol suppression are dependent on the type of preparation, dose and number of injected joints (Armstrong *et al.*, 1981; Knych *et al.*, 2013). Furthermore, splitting the dose between two or more joints may have greater and longer suppressive effect than giving the total dose at one joint. In horses, BTM has shown no detrimental effect when used on carpal joints with osteochondral fragments in exercised horses (Foland *et al.*, 1994). Performance level of exercise in horses may have different rates of systemic absorption of GCs from joints, volumes of distribution and elimination of GCs in comparison with unfit sedentary horses. Such differences may be resultant of differences in the horse body composition and boost in renal and muscle blood flow during exercise (Dulin *et al.*, 2012).

The objectives of our study were first to determine the pharmacokinetics of BTM after a clinically relevant dosage of IA BTM administration in the tarsometatarsal and metatarsophalangeal joints in conditioned exercising horses and secondly, to describe HYD suppression after IA BTM administration.

## MATERIALS AND METHODS

### Horses

Eight female Thoroughbreds (age, mean  $\pm$  SD 4.2  $\pm$  1.6 years; body weight, 483  $\pm$  51.8 kg) were used in the study. An experienced examiner (AB) performed a physical inspection and lameness examination prior to the study; the horses were healthy and free of lameness during the study, and each horse was assessed daily. Three weeks prior to injection, all horses were acclimatized to the stalls and exercised in the high-speed treadmill three times a week (Monday, Wednesday and Friday starting at 9 AM). For each exercise day, the horses underwent walking (9 km/h) for 5 min, followed by trotting (16 km/h) for 5 min, galloping (32 km/h) for 5 min and walking (9 km/h) for 5 min to simulate exercise of race training.

### Experimental design

**BTM administration protocol.** 30 mg (5 mL) of BTM (Celestone Solutspan; Merk & Co., Inc, Whitehouse Station, NJ, USA). One randomly assigned tarsometatarsal joint was injected with 15 mg BMT (2.5 mL); the ipsilateral metatarsophalangeal joint was injected with 15 mg BTM (2.5 mL). Plasma and urine were collected for 6 weeks.

### IA administration of BTM

The horses were sedated with xylazine hydrochloride (Xylazine, 100 mg/mL; Butler Animal Health Supply Co., Dublin, OH, USA) and induced with ketamine hydrochloride (Ketaset; Fort Dodge Animal Health, Madison, NJ, USA). A combination of: xylazine (Xylazine, 100 mg/mL; Butler Animal Health Supply Co.), ketamine (Ketaset; Fort Dodge Animal Health) and guaifenesin (Guaifenesin Injection; Vedco Inc, St Joseph, MO, USA) was used to maintain the horses anesthetized while performing IA injections. The joints were shaved and prepared aseptically. The injections were performed by the same experienced clinician (AB) to maintain consistency, and a successful IA injection was determined through visual detection of clear synovial fluid prior to BTM injection. Synovial fluid collected at injection was submitted for cell count and protein concentration. BTM protocol was injected through a 20-gauge needle. Sides (left or right) were assigned randomly. Horses were not exercised for 3 days after IA injections and then resumed the exercise protocol. Blood samples (16 mL) were collected from the jugular vein into lithium heparin blood tubes prior to the IA administration of BTM (−2, −1 and 0 days) and at 0.25, 1, 2, 3, 4, 6, 8, 10, 12, 24, 36, 48, 72, 96, and 120 h; 7, 10, 14, 21, 25, 27, 29, 32, 34, 36, 39, and 41 days after IA administration. Urine samples (120 mL) were collected in a sterile fashion via urinary catheter, prior to the IA administration of BTM (−2, −1 and 0 h) and at 3, 6, 12, 24, 36, 48, 72, 96, and 120 h; 7, 10, 14, 21, 25, 27, 29, 32, 34, 36, 39, and 41 after IA administration. Blood samples were centrifuged (1500 *g* for 10 min) to separate the plasma. Plasma and urine samples were immediately frozen at −20 °C. Both the blood and urine samples were shipped to the USEF Equine Drug Testing and Research Laboratory (ISO/IEC 17025 accreditation, certificate number 2838.01) for processing. Blood samples were collected between 8:00 and 9:00 AM. Urine samples were collected at the same time for each horse via urinary catheter. The horses were exercised on the high-speed treadmill three times a week based on the schedule described above for the duration of the study.

### Quantitation of BTM and HYD in plasma and urine samples

High-performance liquid chromatography–tandem mass spectrometry methods were used to determine BTM and HYD concentrations in plasma and urine samples (United States Equestrian Federation Equine Drug Testing and Research Laboratory, Ithaca, NY, USA). Plasma and urine aliquots were collected from the middle portion of sample containers and separated on a C18 column (4.6  $\times$  150 mm, 5  $\mu$ m particle size; ZORBAX Eclipse XDB C18 column; Agilent Technologies, Santa Clara, CA, USA) by use of gradient elution with 0.1% formic acid and acetonitrile mobile phases via a high-performance liquid chromatography system (Agilent 1200 HPLC system; Agilent Technologies). Triple quadrupole mass transitions for BTM and HYD were 357.0  $\rightarrow$  135.0 and 363.0  $\rightarrow$  121.0 *m/z*, respectively (Agilent 6410A triple quadrupole liquid chromatography–mass spectrometer; Agilent Technologies). The

quality control (QC) sample average accuracy and precision values included  $R^2 = 0.999987547$  and average QC recovery = 108%. The LLOQs of BTM in plasma and urine samples were 0.05 and 0.25 ng/mL, respectively; LLOQs of HYD in plasma and urine samples were 1 ng/mL.

Concentration–time profiles for BTM and HYD in plasma and urine samples were generated and used for pharmacokinetic description. Noncompartmental pharmacokinetic parameter estimates were initially generated with a computer software program (WinNonlin, version 5.2.1; Pharsight Inc., Mountain View, CA, USA) for plasma and urine BTM concentration vs. time data for each of the eight Thoroughbreds after IA dose of 30 mg of BTM. Linear-up–log–down calculations for AUC were performed by means of two potential cutoff points: last observation or infinity.

Values for quantitative, continuous measurements (e.g., BTM or HYD concentration) and pharmacokinetic parameters (e.g., BTM maximum observed concentration ( $C_{\max}$ ), time of  $C_{\max}$  ( $T_{\max}$ ), terminal-phase elimination rate constant ( $\lambda_z$ ) and time of last quantifiable concentration ( $T_{\text{last}}$ ) for samples collected at each time point for the BTM dose were tested for a normal distribution to ensure appropriate use of parametric statistical methods. Plasma and urine sample HYD concentrations were compared among sampling time points via repeated-measures ANOVA and between baseline and selected postinjection sampling time points via Dunn post-test analysis. Statistical analysis was performed with statistical software (SAS, version 9.2; SAS Institute Inc., Cary, NC, USA). Values of  $P < 0.05$  were considered significant.

## RESULTS

All horses recovered well from anesthesia during the experimental period and completed the study. No lameness or adverse reactions were observed after IA administration of BTM to the horses. All synovial samples collected before IA administration of BTM had total protein concentrations and WBC counts within the reference ranges ( $<2.5$  g/dL and  $<1000$  cells/ $\mu$ , respectively).

### Pharmacokinetics of BTM

Noncompartmental pharmacokinetic parameter estimates for concentrations of BTM in plasma and urine are summarized (Tables 1 & 2). The maximum observed concentration of BTM in plasma ( $C_{\max} = 26.64$  ng/mL) was detected at  $T_{\max}$  of 0.8 h vs.  $C_{\max}$  of 73.5 ng/mL at  $T_{\max}$  of 7.1 h for the urine samples. Time until last quantifiable (i.e., below the LLOQ [0.05 ng/mL] of the assay) plasma BTM concentration ( $T_{\text{last}} = 64.5$  h) was close to urine BTM concentration ( $T_{\text{last}} = 69$  h) (Tables 1 & 2). For the urine samples, AUC of BTM were significantly greater than the plasma samples (Figs 1 & 2).

### Plasma and urine HYD concentrations

Hydrocortisone concentrations were significantly lower in plasma samples collected from horses 1 to 72 h after IA

**Table 1.** Mean  $\pm$  SE noncompartmental pharmacokinetic parameters estimates for BTM in plasma of eight female 4-year-old Thoroughbreds after IA dose of 30 mg of BTM

Parameter	30 mg	
	Mean	SE
$C_{\max}$ (ng/mL)	26.64	4.79
$T_{\max}$ (h)	0.8	0.46
$T_{\text{last}}$ (h)	64.5	6.78
$\lambda_z$ (1/h)	0.0796	0.007
$t_{1/2, \lambda_z}$ (h)	9.22	0.86
$V_z/F$ (L)	2539	217.2
$AUC_{\text{last}}$ (h $\times$ ng/mL)	160.2	15.7

$C_{\max}$ , maximum observed concentration;  $T_{\max}$ , time of  $C_{\max}$ ;  $T_{\text{last}}$ , time of last quantifiable concentration;  $\lambda_z$ , terminal-phase elimination rate constant;  $t_{1/2, \lambda_z}$ , elimination half-life;  $V_z/F$ , observed apparent volume of distribution;  $AUC_{\text{last}}$ , area under the quantifiable concentration–time curve.

**Table 2.** Mean  $\pm$  SE noncompartmental pharmacokinetic parameters estimates for BTM in urine of eight female 4-year-old Thoroughbreds after IA dose of 30 mg of BTM

Parameter	30 mg BTM	
	Mean	SE
$C_{\max}$ (ng/mL)	73.5	9.6
$T_{\max}$ (h)	7.1	1.5
$T_{\text{last}}$ (h)	69	7.1
$AUC_{\text{last}}$ (h $\times$ ng/mL)	1214.4	154.9

$C_{\max}$ , maximum observed concentration;  $T_{\max}$ , time of  $C_{\max}$ ;  $T_{\text{last}}$ , time of last quantifiable concentration;  $AUC_{\text{last}}$ , area under the observed concentration–time curve.

administration of BTM, compared with those in plasma samples collected 2 days, 1 day and before administration of BTM. Hydrocortisone concentrations were significantly lower in urine samples collected from horses 3 to 96 h after IA administration of BTM, compared with those in plasma samples collected 2 days, 1 day and before administration of BTM. The time at which HYD concentration returned to baseline (i.e., before IA administration of BTM) (RTB) in plasma and urine were 96 and 120 h, respectively (Figs 1 & 2).

## DISCUSSION

The purpose of the present study was to determine the pharmacokinetic characteristics of BTM after IA dose of 30 mg of BTM to Thoroughbreds and to describe the effects on endogenous HYD production.

To our knowledge, this is the first study that describes the pharmacokinetics of IA BTM and endogenous hydrocortisone suppression in exercising horses. Foland *et al.* (1994), looked at the effect of BTM and exercise on equine carpal joints with osteochondral fragments. They injected 3.9 mg BTM sodium phosphate and 12 mg BTM acetate. The study looked at the detrimental effects in rested and exercised horses, but pharmacokinetics were not described.

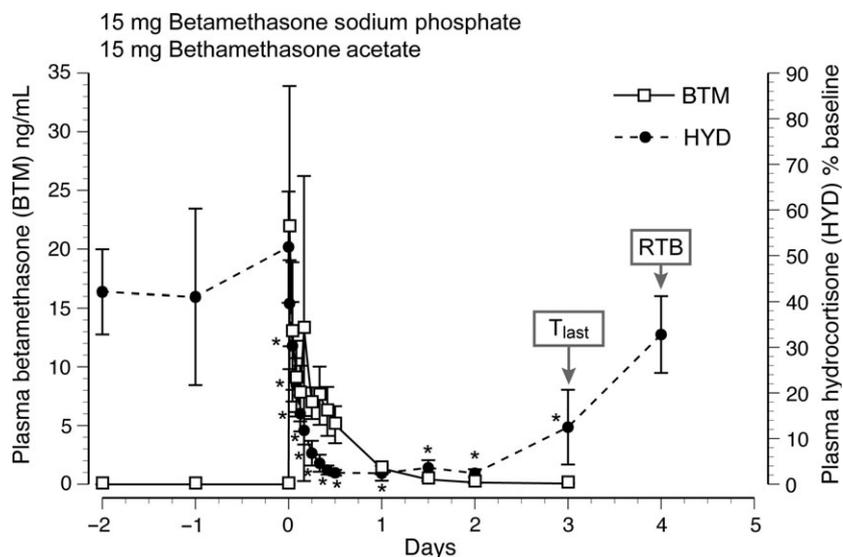


Fig. 1. Mean BTM (white squares; left  $y$ -axis) and HYD (black circles; right  $y$ -axis) concentrations in plasma samples obtained from eight female 4-year-old Thoroughbreds after IA administration of 30 mg of BTM. The HYD concentrations are expressed as percentage relative to the baseline (i.e., before IA administration of BTM) value. Lines of best fit for BTM (solid line) and HYD (dashed line) are indicated. Notice that HYD concentration rapidly decreases after administration of BTM, then increases. \*HYD concentration is significantly ( $P < 0.01$ ) lower than the values before administration of BTM.

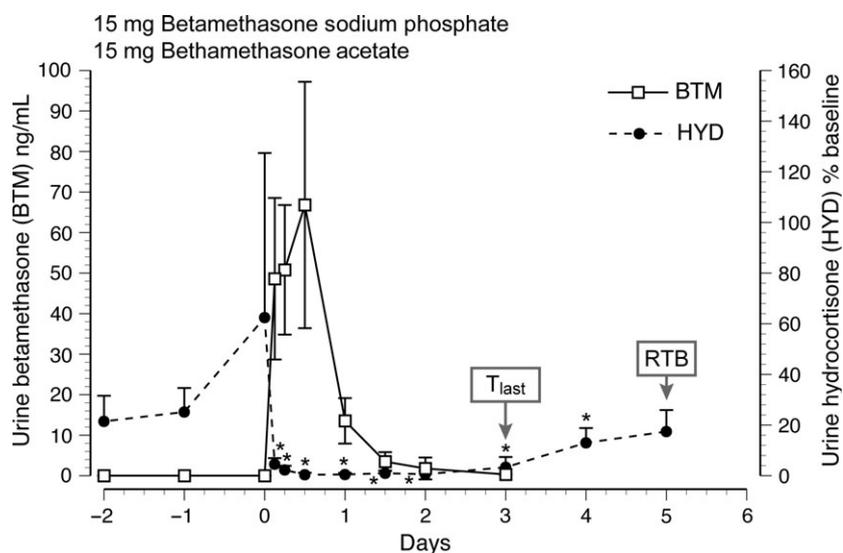


Fig. 2. Mean BTM (white squares; left  $y$ -axis) and HYD (black circles; right  $y$ -axis) concentrations in urine samples obtained from eight female 4-year-old Thoroughbreds after IA administration of 30 mg of BTM. The HYD concentrations are expressed as percentage relative to the baseline (i.e., before IA administration of BTM) value. Lines of best fit for BTM (solid line) and HYD (dashed line) are indicated. Notice that HYD concentration rapidly decreases after administration of BTM, then increases. \*HYD concentration is significantly ( $P < 0.01$ ) lower than the values before administration of BTM.

A faster detection of BTM occurred in plasma in comparison with urine. The time of last quantifiable concentration of BTM was similar in plasma and urine. Use of highly sensitive HPLC-MS/MS assays enabled early detection and prolonged and consistent determination of BTM in plasma and urine. Urine samples showed a greater AUC than plasma, this might be due to urine BTM accumulation in the bladder between samples.

Plasma and urine HYD concentrations were lower than baseline concentrations for 96 or 120 h, respectively. These findings indicated that horses with low or undetectable plasma and/or urine concentrations of HYD could potentially have received exogenous GCs. Further plasma and/or urine sample testing to detect exogenous GCs would be warranted in such cases. These results supported our hypothesis that low plasma and/or urine HYD concentrations could serve as a marker for systemic exposure to exogenous GCs (i.e., BTM). The close relationship between IA administration of BTM and reduced plasma HYD concentrations detected in the present study, and a similar previous study performed by our group determining

the pharmacokinetics of methylprednisolone (MP) and the relationship between MP and hydrocortisone (HYD) concentrations in plasma and urine after intra-articular (IA) dose of 100 or 200 mg in exercised horses (Menendez *et al.*, 2012), was suggestive of a cause and effect relationship. Similar studies with MP showed similar results for the decreased plasma concentrations of endogenous GCs following IA administration of MP (Autefage *et al.*, 1986; Soma *et al.*, 2006, 2011).

The decrease in HYD concentration in plasma and urine revealed a suppression of the hypothalamus–pituitary–adrenal axis and the production of HYD. The suppression of HYD in plasma and urine was maintained for 96 and 120 h, respectively. These results in conjunction with the results showed in similar MP studies (Autefage *et al.*, 1986; Soma *et al.*, 2006, 2011; Menendez *et al.*, 2012) confirmed that horses who received IA GCs administration (BTM and MP) had significant effects on HYD suppression. Thus, suppression of endogenous cortisol production may have important implications for establishing withdrawal times following administration of synthetic

GCs. Further research is crucial to determine whether measurement of plasma HYD concentrations can be used as a screening tool for identification of horses that received exogenous GCs. For a study on dose relationships, it was critical to be certain that all of the drug went into the joint. This was considered a risk in a standing horse, even with an experienced clinician performing the injections. We tried to avoid the added variable of animals moving during injection or flexing the joint during injection or peri-articular loss of injective. Simultaneous administration of sedatives for joint injections, are routinely given for standing injections. We wanted to verify that all the injection went into the joints. The influence of sedatives on the BTM pharmacokinetics is unknown.

The recommended withdrawal for BTM according to USEF is 7 days (2014 USEF Rule Book). It is important to reiterate that the formulation of the drug, dose administered, the frequency of administration, the route, the joints, and the condition of the horse, greatly affect the threshold concentration.

Because of the modest dataset (eight horses) and experimental procedures used (treadmill exercise) in the present study, extrapolation of our findings to larger populations of sport and racing horses in competition should be performed with caution.

Results of the present study described the BTM pharmacokinetics and shown a faster detection of BTM in plasma than urine. The time of last quantifiable concentration of BTM was similar in plasma and urine. The use of highly sensitive HPLC-MS/MS assays enabled early detection and prolonged and consistent determination of BTM in plasma and urine.

Plasma and urine HYD concentrations would rapidly decrease after IA administration of BTM. Further research is imperative to validate the use of plasma HYD concentrations as a screening tool for identification of horses that received exogenous GCs.

#### ACKNOWLEDGMENTS

The authors thank Drs. Stephen Schumacher, Thomas Lomangino, Emily Falk, Michelle Bacha; Carissa Norquest, Kristen Zucco, Leanne Diese, Natalee Ziegler, and Rebekah Sanchez-Hodge for technical assistance; Dr Akikazu Ishihara for statistical assistance, and Tim Vojt for assistance with preparation of illustrations.

#### SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

**Data S1** Quantitation data analysis.

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