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JOURNAL: EQUINE VETERINARY JOURNAL

PUBLISHER: Wiley

PUBLICATION DATE: 8 October 2018 (online)

DOI: https://doi.org/10.1111/evj.13031

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Article type : General Article

EVJ-GA-18-060.R2

Characterisation of the *in vivo* interactions between detomidine and methadone in horses: pharmacokinetic and pharmacodynamic modelling

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Running title: Pharmacokinetic/pharmacodynamic modelling of detomidine/methadone in horses.

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This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/evj.13031

Summary

Background: Pharmacokinetic (PK)/pharmacodynamic (PD) modelling offers new insights to design protocols for sedation and analgesia in standing horses.

Objectives: To evaluate the parameters and interactions between detomidine and methadone when given alone or combined in standing horses.

Study design: Randomised, placebo-controlled, blinded, crossover.

Methods: Eight adult healthy horses were given six treatments intravenously: saline (SAL); detomidine (5 µg/kg bwt; DET); methadone (0.2 mg/kg bwt; MET) alone or combined with detomidine [2.5 (MLD), 5 (MMD) or 10 (MHD) µg/kg bwt]. Venous blood samples were obtained at predetermined times between 0 and 360 minutes after drug administration. Plasma detomidine and methadone were measured using a single, liquid/liquid extraction technique by liquid chromatography coupled with a triple quadrupole mass spectrometer (LC-MS/MS). Sequential PK/PD modelling compared rival models, with and without PK and PD interaction between drugs, to fit the PD data including height of the head above the ground (HHAG), a visual analogue scale for sedation (VAS), electrical (ET), thermal (TT) and mechanical (MT) nociceptive thresholds and gastrointestinal motility (GIM) [1].

Results: Two and three compartment models best described the PK of detomidine and methadone, respectively. Detomidine decreased its own clearance as well as the clearance of methadone. The interaction of methadone on the effect of detomidine revealed an infra-additive effect for HHAG (α = - 1.33), VAS (α = - 0.98) and GIM (α = - 1.05), a positive potentiation for ET (pot = 0.0041) and TT (pot = 0.133) and a synergistic to additive effect for MT (α = 0.78).

Main limitations: This is a small experimental study.

Conclusions: Different PK/PD interactions were demonstrated for each PD parameter and could be modelled *in vivo*. The modelling of our data will allow us to simulate and predict the effect of constant rate infusions of both drugs for future investigations.

Introduction

Single intravenous (i.v.) combinations of α_2 adrenoceptor agonists and opioids are commonly used in standing horses to provide or enhance sedation and analgesia. Detomidine has been extensively used in this way in combination with several different opioids [2-5] including methadone [2]. The pharmacodynamics (PD) of a number of i.v. combinations of detomidine with methadone were recently studied in order to identify a low dose combination that produces antinociception with minimal adverse effects [1].

The pharmacokinetics (PK) of high i.v. doses of detomidine (30 µg/kg bwt) [6-8] and methadone at clinical doses (0.15 mg/kg bwt) [9] have been studied when administered separately. Sequential PK/PD modelling with nonlinear mixed effect models (NLME) is well established in the literature [10-12] and aims to report the relationship between concentration-timecourse and effect of one or several drugs in a single model. This modelling approach is required to describe accurately what happens in terms of drug interaction. Furthermore, modelling allows simulation of an unobserved scenario, with other dose rates than those tested. In horses, these methods were used to model the PK and PD of detomidine 30 µg/kg bwt after i.v. or intramuscular administration [13]. Such PK/PD modelling of methadone in horses has not been reported, either alone or in combination with detomidine. Similarly, neither the PK nor any PK/PD modelling has been reported in horses after α_2 adrenoceptor agonist-opioid combination administration in conscious horses.

Therefore, the aim of the investigation was to develop a single analytical technique that would determine both detomidine and methadone in plasma samples, and model the PK and PD [1] data to characterise their interactions when given alone or in combination. An experimental study was designed to estimate the PK parameters in a six-period crossover including i.v. saline, detomidine (5 µg/kg bwt) alone and methadone (0.2 mg/kg bwt) alone, or combined with 2.5, 5 or 10 µg/kg bwt detomidine, and to model the interactions between their plasma concentration-timecourses and their PD effects [1].

Methods

This investigation was designed as a randomised, placebo-controlled, observer-blinded, crossover study, with a washout period of at least one week between each of the six different treatments.

Animals

Plasma samples and PD data collected from the eight healthy adult crossbred horses studied by Gozalo-Marcilla *et al.* [1] were included in the study. They comprised four castrated males and four females aged 7 ± 2 years, weighing 372 ± 27 kg. A test of power of 80% and a significance level of 5% were used [1]. No sedatives or analgesics were administered for at least one month before the study.

Drug administration

After skin disinfection and subcutaneous injection of 1 ml 2% lidocaine (Xylestesin 2%[a]), two 14 gauge i.v. catheters were placed in the direction of the blood flow, one in the right jugular vein for drug administration (BD Angiocath 14 GA x 1.88[b]), and the other in the left jugular vein for blood sampling (G14 x 70 mm[c]). The final volume of injection was adjusted to 10 ml by adding saline and administered manually over 10 seconds at T0 by an assistant investigator (N.C.).

In addition to saline control, five treatments were administered: 5 µg/kg bwt detomidine (Eqdomin 10 mg/ml[d]) (DET), 0.2 mg/kg bwt methadone (Mytedom 10 mg/ml[a]) alone (MET), or combined with 2.5 (MLD), 5 (MMD) or 10 (MHD) µg/kg bwt detomidine.

Measurements and sample collection

Pharmacodynamic variables (see below) were measured in triplicate by the main investigator (M.G.M.) at baseline before T0 and at 5, 15, 30, 45, 60, 75, 90, 120 and 180 min [1]. The main investigator was unaware of the identity of the treatment. Blood samples were taken by the assistant investigator 3 min before T0 and at 3, 5, 15, 30, 45, 60, 75, 90, 120, 180, 240, 300 and 360 min after drug administration. Blood was withdrawn at each time point

immediately after assessment of sedation and cardiovascular evaluation and before nociceptive threshold testing and gastrointestinal data collection [1].

Prior to drawing each 10 ml blood sample, another 10 ml of blood were discarded from the left jugular catheter. After each blood sampling, the catheter was flushed with 10 ml of a dilute heparinised saline solution (10 IU/ml). Blood samples were collected into heparinised blood tubes, labelled with the horse's identification number and the sample time point and stored at 4 degrees Celsius (°C) for a maximum of 90 min before centrifugation at 1330 g (Combate centrifuge[e]). The plasma was then transferred into previously labelled storage cryovials and stored at -70°C until PK analysis.

Sample preparation

Before sample preparation for PK analysis, plasma from the horses in the saline treatment group was used to validate a single method for quantification of both detomidine and methadone, which is described below.

A volume of 290 µl of thawed plasma was combined with 10 µl of the internal standard (IS) fentanyl (30 ng/ml) in an Eppendorf Safe-Lock tube. A liquid/liquid extraction technique was employed by using 1 ml of ethyl acetate. After mixing for 15 min in a rotary shaker at 1000 rpm (rotary shaker AV-2[f]), the sample was centrifuged for 10 min at 11180 g at 4°C (M-240R centrifuge[g]). Later, 800 µl of the supernatant were transferred into another Eppendorf Safe-Lock tube and evaporated for 40 min at 30°C (RVC 2-18 speed-vacuum[h]). The samples were immediately reconstituted in 100 µl of the liquid chromatography mobile phase, mixed for 1 min (rotary shaker AV-2[f]) and centrifuged for 5 min at 11180 g at 4°C. Finally, 80 µl of the supernatant were transferred to a new vial.

Determination of detomidine and methadone concentrations

The samples were analysed by liquid chromatography[i] coupled with a triple quadrupole mass spectrometer (Applied Biosystems/Sciex API 3200[j]) (LC-MS/MS). Chromatographic separation was achieved on a C_{18} column (C18 Kinetex 100 x 2.1 mm; 2.6 µm[k]) with a pre-column of the same material, maintained at 40°C, with eluent A (ammonium acetate 5 mM + acetic acid 0.1%) and eluent B (acetonitrile + acetic acid 0.1%), used in a gradient mode (Supplementary Item 1). The volume of injection was 4 µl and the flow rate 0.3 ml/min. Retention times for detomidine, IS and methadone were 1.6, 2.8 and 3.9 min, respectively.

The MS was operated in the positive ion and multiple reaction monitoring (MRM) mode for the transitions 187→81 (detomidine), 310→105 (methadone) and 337→105 (fentanyl). Lower limits of quantification (LLOQ) were 0.10 and 0.05 ng/ml for detomidine and methadone, respectively.

Pharmacokinetic analysis

The plasma concentrations of both drugs at each time point were analysed with NLME using compartmental modelling with a physiologically relevant parametrisation (Phoenix 8.0[l]).

Plasma concentration time curves for each drug were explored using one, two and three compartment models, defined by the clearance (Cl) and volumes of distribution for the central (V_1) and peripheral compartments (V_2, V_3). For each PK parameter, individual values are modelled as functions of population typical value (tv) and individual random deviations Eta (η). Distributions for V and Cl for each drug for each compartment were fitted according to a log normal distributions, where for the i^{th} individual, $V_i = tv_V * \exp(\eta V_i)$ and $Cl_i = tv_{Cl} * \exp(\eta Cl_i)$, respectively, with η following a normal distribution centred on 0 and with a variance ω^2. A parsimonius selection method to justify the progressive inclusion of additional random effects was used to ensure the individual parameters were identifiable. Secondary parameters including volume of distribution at steady state (Vdss) and half lifes ($t_{1/2\alpha}$, $t_{1/2\beta}$, $t_{1/2\gamma}$) were calculated as secondary parameters in Phoenix[l].

The plasma concentration-time curves were initially fitted individually for each drug before modelling the two PK datasets together. Preliminary non-compartmental analysis (Phoenix 8.0[l]) revealed non-linearity in the detomidine PK (supra proportional increase in the area under the curve as the dose increases) and that detomidine significantly reduced the Cl of methadone. According to our hypothesis, we added a function to encode the modulation of detomidine and methadone Cl by detomidine in the PK model (proportionally to the concentration or the log of the concentration of detomidine).

Pharmacodynamic data

The following data, as reported by Gozalo Marcilla *et al.* [1], were included in the PD model: height of the head above the ground (HHAG, cm), the visual analogue scale for sedation (VAS, 0 to 10 score, 0 no sedation neither ataxia, 10 maximal sedation and ataxia), nociceptive thresholds for electrical [ET, cut-out 20 Volts (V)], thermal (TT, cut-out 60ºC) and mechanical [MT, cut-out 20 Newtons (N)] stimuli, and gastrointestinal motility (GIM, 0 to 20 score, 0 no intestinal sounds in any of the four abdominal quadrants, 20 long, loud, gurgling sounds, with a frequency of more than four sounds per min).

Pharmacokinetic/pharmacodynamic model selection criteria

Sequential PK/PD modelling (after freezing the PK parameters), was used to compare suitability of rival PK/PD models, with and without PD interaction between drugs, for fitting the PD data and estimating negative hysteresis. The plots were evaluated visually to determine the best model; these featured the observed data *versus* individual and population predictions, individual and population weighted residuals *versus* time and conditional weighted residuals *versus* time. The value of the Objective Function (OVF = -2 Log likelihood) and the Akaike Information Criterion (AIC) [14] were also used for model selection. Finally, visual predictive checks (VPCs) compared (by estimation of overlap) the 20^{th}, 50^{th} (median) and 80^{th} percentile predictions generated by the models to the distribution of the observed data.

Negative hysteresis (delay between plasma concentration and effect) was modelled with an effect compartment model. The link between the predicted plasma concentrations (*Cp*) and the effect compartment concentration (*Ce*) was given by the equation 1:

$$\frac{dCe}{dt} = K_{e0} \times (Cp - Ce) \quad (1)$$

with k_{e0} being the transfer rate constant between the central compartment and the effect compartment (1/h). Pharmacodynamic endpoints were modelled independently from each other, therefore each candidate PK/PD model included two residual proportional error terms for PK (one per drug) and one additional error for the PD fitting. Only plasma concentrations equal to or above the LLOQ for the assays were included in the analysis.

Results

All the animals tolerated the experiments and the study was conducted without complications. Only one horse became deeply sedated when receiving both drugs, alone or in combination [1].

Pharmacokinetics

The LC-MS/MS method for quantification of detomidine and methadone was validated according to the Guideline on bioanalytical method validation [15]. All validation data (precision, accuracy and stability) are reported in Supplementary Item 2.

The plasma concentration-time curves for the two drugs are shown in Figs 1a and 1b. A two-compartment model with linear interaction best described the PK of detomidine (Figs 1 and 2). Detomidine decreased its own Cl in a concentration-dependent fashion (decreased OVF: 204). The maximal detomidine body Cl was 2.63 l/kg bwt/h and it was reduced by the product of the coefficient of moderation of detomidine Cl (called S = 0.049) and the plasma concentration of detomidine. For illustration, this effect decreased Cl by 3.8, 1.5, 1% at 30 min, 1 and 2 hours, respectively after administration of MMD. Pharmacokinetic values for both drugs are shown in Table 1.

A three-compartment model best fitted the PK of methadone (Figs 1 and 2). Inclusion of a detomidine concentration-dependent effect on methadone Cl significantly improved the fit. The methadone Cl was 0.49 l/kg bwt/h and was reduced by the product of a coefficient describing the moderation of methadone Cl by detomidine ($p = 0.063$) and the detomidine plasma concentrations (e.g. 24, 2.0, 1.1% reductions at 30 minutes, 1 and 2 hours, respectively after administration of MMD).

Pharmacokinetic-pharmacodynamic modelling

Individual PK parameters (typical values tv and individual random deviations Eta η) of the best PK model were fixed to estimate the PD values in a sequential PK/PD modelling process. Seven rival PD models were compared to identify the best relationship between the

plasma concentrations and their potential interactions with the evaluated PD values. These models included the effect of one or both drugs and are shown in Supplementary Item 3.

The terms used to describe the PD interactions are the following, depending if methadone has its own effect (alone) in the final model (then the interaction is either synergism, additivity or infra-additivity/antagonism), or not (positive of negative potentiation).

-Additivity, i.e. 1 + 1 = 2, the effects of the two drugs in one PD parameter are the same as the sum of the individual effects; as Borrat *et al.* [16] with $\alpha = 0$.

-Synergism, i.e. 1 + 1 > 2, the effects of the two drugs in one PD parameter are more than additive; as Borrat *et al.* [16] with $\alpha > 0$.

-Infra-additivity (or partial antagonism), i.e. 1 + 1 < 2: the effect of one drug is decreased or suppressed by another drug; as Borrat *et al.* [16] with $\alpha < 0$.

-Positive potentiation, i.e. 1 + 0 > 1, the effect of one drug is increased by the administration of another drug itself without effect; pot > 1.

The parameters for the PD variables are shown in Table 2. Overall PK and PD results are summarised in Tables 3 and 4.

The VPC plots for HHAG, VAS, ET, TT, MT and GIM are shown in Figures 3a, 3b, 3c, 3d, 3e and 3f, respectively.

Discussion

This investigation makes an important contribution to clinical equine science by using PK/PD modelling data to describe the *in vivo* interaction between two drugs at six different endpoints. Equine plasma samples were analysed with an improved PK analytical method designed to measure concentrations of both α_2-agonists and opioids simultaneously, reducing extraction times and costs dramatically. This single method for determination of both drugs was developed from previous studies of detomidine [6-8,13] and methadone [9,18]. As also reported by Knych *et al.* [8] and Grimsrud *et al.* [13], a two-compartment model best described the PK of detomidine in equine plasma in our study. However, for methadone, a

three-compartment model was used to describe its behaviour in contrast to previous reports of a two-compartmental best fit [9,18]. The PK data evaluation demonstrated detomidine's inhibitory effect on methadone's and on its own Cl, in agreement with observations on dexmedetomidine in humans [19] and cats [20]. In those studies, the α_2 agonist reduced in its own drug elimination Cl directly proportional to the effects on cardiac output. This relationship cannot be confirmed in our study as the protocol was not intended to evaluate the cardiopulmonary effects, so cardiac output was not measured.

The second impact of our methodology relates to refinement of the modelling phases. The PD data collected by Gozalo-Marcilla *et al.* [1] are measures of sedation (HHAG and VAS), antinociception (increased ET, TT and MT), and the side effect of intestinal hypomotility (GIM). These end-points are established representatives of the expected clinical effects ideally suited to PK/PD analysis. Our PK/PD modelling with NLME models allows information leverage and pooling from different periods of the study to identify shared PK and PD parameters [21]. Moreover, it enabled missing values and inconsistencies of the dataset to be managed. For example, one of the horses was profoundly sedated by both drugs, either alone or in combination [1]; but this was consistent with his abnormal PK.

The simultaneous use of sedatives and analgesics is common practice in veterinary medicine, and it is generally assumed that drugs are additive. However, this may not always be the case, depending on different PD variables and physiological responses. In horses, it is widely accepted that combinations of opioids with α_2 agonists enhance the sedative and analgesic properties of the latter [2,4,5]. Previously, no studies have explored the potential interactions between these agents in detail, expressed by empirical models using PD effects and plasma concentrations. The complex models for synergism [16,17] and potentiation allowed extraction of as much information as possible from our data collected from a small sample of the population, including evaluation of between-drug interaction. Future research using similar study designs would benefit from the same approach.

An infra-additive synergism (or partial antagonism) was demonstrated for both measures of sedation, HHAG and VAS. With detomidine alone, HHAG was reduced, whereas methadone had a minimal or no effect on its own. However, the combination of methadone with detomidine partially reversed the detomidine-induced reduction in HHAG [1] due to this infra-additive synergism (higher HAAG values). Opioids may produce excitement and increased locomotor activity, especially at high doses [22]. At clinical doses,

Oliveira *et al.* [23] reported that the chin-to-floor distance decreased for 30 min after i.v. detomidine alone (10 µg/kg bwt) or combined with methadone (0.2 mg/kg bwt), but with the combination treatment the horses' heads were higher for the first 5 min. Oliveira *et al.*'s conclusion that "methadone did not produce a synergic or additive sedative effect when combined with detomidine" is consistent with our results, indicating that this interaction can be defined as a negative potentiation [23]. A similar reasoning applies to the subjective measurement of sedation, VAS.

A number of interactions were detected by evaluation of the antinociceptive effects: synergism to additivity was demonstrated with MT, whereas ET and TT showed potentiation. According to this analysis, methadone itself did not produce any electrical or thermal antinociception, but it potentiated the effects of detomidine [1]. The model indicated that methadone alone produced limited mechanical antinociception (none in some individuals). This may be a result of the different fibres activated by the stimuli employed (i.e. electrical - nociceptive Aδ and C, and the Aβ not directly involved in antinociception; thermal - mainly C fibres; mechanical - both Aδ and C) [24]. This demonstrates the importance of using more than one stimulus when performing antinociceptive studies in standing horses [25]; agents from dissimilar drug groups, such as α_2 agonists and opioids, may have different mechanisms of action.

For GIM, the drug combination produced an infra-additive effect. It is well known that α_2 agonists [26,27] and opioids [22,28] decrease intestinal motility. Although detomidine and methadone both have this effect when administered alone, it was not additive according to the model that best fitted the GIM data. Indeed, as demonstrated by Gozalo-Marcilla *et al.* [1], addition of 0.2 mg/kg bwt methadone to 5 µg/kg bwt detomidine (MMD) did not change the GIM scores recorded after detomidine alone (DET, 5 µg/kg bwt).

All the models predicted the behaviour of the various parameters for all treatments with the exception of MLD. With MLD, the PK model tended to overestimate detomidine concentrations and therefore the sequential PK/PD modelling overestimated the effect of MLD for some of the endpoints. This may be explained by the minimal, short-term effects produced by this combination. It could be argued that a lower LLOQ than 0.10 ng/ml should have been used, in order to detect detomidine concentrations for longer, as reported by Grimsrud *et al.* [6] and Knych *et al.* (2012) [8]. A lower LLOQ would probably have allowed

better modelling for this treatment (MLD). However, this is of limited importance as this combination produced no sedation and minimal antinociception [1], making MLD of limited clinical value.

The main advantage of using sequential PK/PD modelling is the ability to simulate the effect of different dosage regimens in order to find the optimal one to be tested under experimental circumstances. For example, with our data, we were able to simulate different protocols of combined i.v. *boli* and constant rate infusions (Supplementary Item 4) for the design of our next experimental study [29] before using these optimised protocols in a clinical study. However, we can only simulate within the range of doses tested and the associated plasma concentrations; modelling outside those ranges may lead to false predictions and misleading conclusions. A second limitation is the absence of different doses of methadone. Inclusion of more than one dose would have allowed more detailed study of the drug interactions. Nevertheless, it would have been technically challenging to perform a nine or sixteen period crossover study with four doses of both detomidine and methadone. A final limitation refers to the analytical method for methadone, as it was not enantiomer-selective. Reported concentrations correspond to levo- and dex-methadone. Consequently, PK/PD modelling was performed assuming the same potency for both enantiomers.

In conclusion, this study demonstrates the optimal use of PK/PD data from a six-period crossover study for estimating the PD and the interaction parameters for six different endpoints. It describes the PK and PD interactions of detomidine and methadone when given alone or in combination, which are different for each PD parameter: negative potentiation for HHAG, positive potentiation for TT and ET, infra-aditivity for VAS and GIM and synergism for MT. The resulting model will be used to predict the effect of constant rate infusions of both drugs in planning future investigations.

Authors' declaration of interests

No competing interests have been declared.

Ethical animal research

The study was approved by the Local Ethical Committee of the Faculty of Veterinary Medicine and Zootecnia (FMVZ) of the State of São Paulo University (UNESP), Botucatu, Brazil (08/2015).

Source of Funding

São Paulo Research Foundation (FAPESP), scholarship grants numbers (2010/08967-0, 2014/00474-5, 2014/50265-3 and 2017/01425-6).

Acknowledgements

The authors would like to thank the pharmaceutical company Ourofino by the donation of the injectable form and the active form of detomidine and Cristalia by the donation of the active form of methadone (Agência Nacional de Vigilância Sanitária number 25351.049229/2016-51).

Authorship

M. Gozalo-Marcilla contributed to study design, acquisition, management and interpretation of data and preparation of the manuscript. S. Luna contributed to study design, interpretation of data and review of the manuscript. R. Moreira da Silva contributed to the acquisition, management and interpretation of data and preparation of the manuscript. N. Crosignani contributed to study design and acquisition of data. N. Lopes contributed acquisition, management and interpretation of data. P. Taylor contributed to study design and critically

reviewed the manuscript. L. Pelligrand contributed to study design, management and interpretation of data, preparation and critical review of the manuscript. All authors approved the final manuscript.

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Supporting Information

Supplementary Item 1: Gradient mode used in the liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) system.

Supplementary Item 2: Validation parameters for a single method used to detect simultaneously detomidine and methadone in horse plasma.

Supplementary Item 3: Pharmacodynamic (PD) rival models identifying the best relationships between the detomidine and methadone plasma concentrations and their potential interactions.

Supplementary Item 4: Simulations of the pharmacodynamic (PD) effects of detomidine and methadone.

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		Detomidine				Methadone		
Parameter	**Unit**	**Estimate**	**ηshk**	**IIV%**		**Estimate**	**ηshk**	**IIV%**
tvCl1	l/kg bwt/h	**2.63**	**6%**	**1.9**		**0.490**	**3%**	**2.8**
tvCl2	l/kg bwt/h	**0.953**	**-**	**-**		**3.16**	**-**	**-**
tvCl3	l/kg bwt/h					**0.249**	**-**	**-**
tvV1	l/kg	**0.618**	**-**	**-**		**0.102**	**-**	**-**
tvV2	l/kg	**0.724**	**-**	**-**		**0.310**	**11%**	**5.9**
tvV3	l/kg					**0.218**	**9%**	**30.3**
tvS	l/µg	**0.049**	**28%**	**2.9**				
tvp	l/µg					**0.063**	**22%**	**11.7**
r.e. $_{PK}$		**38%**				**26%**		
vdss	l/kg	**1.34**				**0.77**		
$t_{1/2\,\alpha}$	h	**0.11**				**0.02**		
$t_{1/2\,\beta}$	h	**0.71**				**0.38**		
$t_{1/2\,\gamma}$	h					**1.62**		

Table 1: Typical values (tv) and Inter Individual Variability (IIV) of pharmacokinetic parameters of detomidine and methadone in 8 standing horses following 5 treatments administered intravenously: 0.2 mg/kg bwt methadone (MET), 5 µg/kg bwt detomidine alone (DET), or 0.2 mg/kg bwt methadone combined with 10 (MHD), 5 (MMD) or 2.5 (MLD) µg/kg bwt detomidine. The reported parameters included clearance (Cl), volumes of distribution for the central (V1) and peripheral compartments (V2, V3), S: coefficient of moderation of detomidine clearance by detomidine, p: coefficient of moderation of methadone clearance by detomidine. ηshk: eta shrinkage (%), the parameters' IIV% is only reported when shrinkage is acceptable (<30%), i.e when there is enough data to identify individual parameters. r.e.$_{PK}$: residual error of the PK model, vdss: volume of distribution at steady state, and $t_{1/2}$: distribution or elimination half-lives. Omega is the variance of the PK parameters.

		HHAG (cms)			VAS (0 – 10 score)			MT (Newtons)			TT (°Celsius)			ET (Volts)			GIM (0 – 20 score)		
Parameter	**Units**	**Est.**	**ηshk**	**IIV%**	**Est.**	**ηshk**	**IIV%**	**Est.**	**ηshk**	**IIV%**	**Est.**	**ηshk**	**IIV%**	**Est.**	**ηshk**	**IIV%**	**Est.**	**ηshk**	**IIV%**
tvK_{e0}_DET	**1/h**	**5.3**	**-**	**-**	**8.5**	**5%**	***15.9***	**8.6**	**3%**	***23.3***	**8.2**	**-**	**-**	**8.6**	**12%**	***38.1***	**5.6**	**-**	**-**
tvK_{e0}_MET	**1/h**	**8.9**	**-**	**-**	**3.2**	**-**	**-**	**2.2**	**9%**	**133**	**39.5**	**-**	**-**	**14.6**	**-**	**-**	**8.9**	**-**	**-**
E_0 (baseline)	*	**98.8**	**2%**	**0.28**	**0**	**-**	**-**	**0.8**	**9%**	***59.1***	**44.0**	**3%**	***0.19***	**1.7**	**-**	**-**	**14.7**	**-**	**-**
tvI_{max} combination	*	**1**	**(fixed)**														**1**	**(fixed)**	
tvIC_{50}DET	**ng/ml**	**3.8**	**3%**	**46.0**													**2.5**	**19%**	***3.5***
tvIC_{50}MET	**ng/ml**	**846**	**-**	**-**													**1010**	**24%**	***31.4***
tvE_{max} combination	*	**-**	**-**	**-**	**8.4**	**8%**	***1.7***	**19.1**	**-**	**-**							**-**	**-**	**-**
tvEC_{50}DET	**ng/ml**	**-**	**-**	**-**	**2.39**	**4%**	***9.6***	**4.15**	**25%**	***1.4***							**-**	**-**	**-**
tvEC_{50}MET	**ng/ml**	**-**	**-**	**-**	**421**	**10%**	***9.6***	**401**	**13%**	***12.1***							**-**	**-**	**-**
tvn_DET	**-**	**1.36**	**9%**	***21.1***	**1.89**	**-**	**-**	**15.5**	**-**	**-**							**2.07**	**8%**	***21.8***
tvn_MET	**-**	**6.6**	**8%**	***23.7***	**18.2**	**7%**	***13.9***	**2.2**	**-**	**-**							**0.89**	**11%**	***34.3***
α (synergism)	**-**	**-1.33**	**10%**	***15.2***	**-0.98**	**21%**	***6.0***	**0.78**	**11%**	***251***							**-1.05**	**24%**	***22.9***
Borrat model type:		***Infra-additive***			***Infra-additive***			***Synergistic/Additive***									***Infra-additive***		

Potentiation model:													***[Methadone]***			***log [Methadone]***					
tvE_{max} DET alone	*												**10.0**	-	-	**3.1**	**18%**	***56.8***			
tvEC_{50}DET	**ng/ml**												**2.1**	**16%**	***9.0***	**3.9**	**7%**	***69.3***			
tvn_DET	-												**1.94**	**20%**	***13.1***	**2.38**	**26%**	***14.9***			
pot (potentiation)	**ml/ng**												**0.133**			**0.0041**	**20%**	**130**			
r.e_{PD}	*	**6.97**				**0.49**			**1.91**				**3.28**			**0.74**			**1.51**		

Table 2: Pharmacokinetic/pharmacodynamic parameters of detomidine and methadone in 8 standing horses following 5 treatments administered intravenously: 0.2 mg/kg bwt methadone (MET), 5 µg/kg bwt detomidine alone (DET), or 0.2 mg/kg bwt methadone combined with 10 (MHD), 5 (MMD) or 2.5 (MLD) µg/kg bwt detomidine. Pharmacodynamic variables include the height of the head above the ground (HHAG), gastrointestinal motility (GIM), the visual analogue scale (VAS), and antinociceptive thresholds with mechanical (MT), thermal (TT) and electrical (ET) stimuli. T HHAG, VAS and GIM best fitted infra-addition (1 + 1 < 2), whereas MT better synergism (1 + 1 > 2), both using the model from Borrat *et al.* [16]. The TT and ET best fitted an empirical positive potentiation model (1 + 0 > 1). *tvKe0_DET* is the typical value used to predict the dose-concentration relationship and the course of the effect of detomidine, *tvKe0_MET* is the typical value used to predict the dose-concentration relationship and the course of the effect of methadone, *E0* is the response at baseline. *tvImax combination* is the typical value of the maximal response of the combination of drugs, *tvIC50DET* is the typical value of the effect site concentration eliciting a response equal to half of the baseline response with detomidine, *tvIC50MET* is the typical value of the effect site concentration eliciting a response equal to half of the baseline response with methadone, *tvEmax combination* is the typical value of the maximal effect of the combination of drugs, *tvEC50DET* is the typical value of the effect site concentration eliciting a response equal to half of *E0* with detomidine, *tvEC50MET* is the typical value of the effect site concentration eliciting a response equal to half of E0 with methadone, *tvn_DET* is the typical value for n which determines steepness of the curve of the parameter *versus Ce* methadone curve, *α (synergism)* is the interaction parameter, *tvEmax DET alone* is the typical value of the maximal effect of detomidine alone, *tvn_MET* is the typical value for n which determines steepness of the curve of the parameter *versus* the predicted effect site concentration (*Ce*) methadone curve, *pot (potentiation)* is the potentiation parameter. ηshk: eta shrinkage (%), the parameters' IIV% is only reported when shrinkage is acceptable (<30%), i.e when there is enough data to identify individual parameters. $r.e._{PD}$: residual error for each PD endpoint.

* unit depends on PD endpoint, HHAG (cms), VAS (0 – 10 score), MT (Newtons), TT (°Celsius), ET (Volts), GIM (0 – 20 score); IIV %: interindividual variability (expressed as a percentage coefficient of variation).

Effect of	Parameter affected	Overall effect	Comment
Plasma detomidine concentration	Detomidine clearance	Decrease	Detomidine inhibits its own clearance (supposedly through reduction of cardiac ouput)
Plasma detomidine concentration	Methadone clearance	Small decrease	Detomidine inhibits methadone´s clearance (supposedly through reduction of cardiac ouput)

Table 3: Summarised pharmacokinetic results of the effects of detomidine and methadone in 8 standing horses following 5 treatments administered intravenously: 0.2 mg/kg bwt methadone (MET), 5 µg/kg bwt detomidine alone (DET), or 0.2 mg/kg bwt methadone combined with 10 (MHD), 5 (MMD) or 2.5 (MLD) µg/kg bwt detomidine.

Parameter	Effect of detomidine alone	Effect of methadone alone	Effect of interaction	Model	Value of interaction parameter
HHAG (cms)	↓↓	= or ↓	**Infra-additive (antagonism)**	$E = E_0 \times (1 - I_{max} \times \frac{\left(\frac{C_eDet}{IC_{50}Det} + \frac{C_eMet}{IC_{50}Met} + \alpha \times \frac{C_eDet}{IC_{50}Det} \times \frac{C_eMet}{IC_{50}Met}\right)^{nDet,Met}}{1 + \left(\frac{C_eDet}{IC_{50}Det} + \frac{C_eMet}{IC_{50}Met} + \alpha \times \frac{C_eDet}{IC_{50}Det} \times \frac{C_eMet}{IC_{50}Met}\right)^{nDet,Met}}$	α = - 1.33
VAS (0 – 10 score)	↑↑	↑	**Infra-additive (antagonism)**	$E = E_0 + E_{max} \times \frac{\left(\frac{C_eDet}{EC_{50}Det} + \frac{C_eMet}{EC_{50}Met} + \alpha \times \frac{C_eDet}{EC_{50}Det} \times \frac{C_eMet}{EC_{50}Met}\right)^{nDet,Met}}{1 + \left(\frac{C_eDet}{EC_{50}Det} + \frac{C_eMet}{EC_{50}Met} + \alpha \times \frac{C_eDet}{EC_{50}Det} \times \frac{C_eMet}{EC_{50}Met}\right)^{nDet,Met}}$	α = - 0.98
MT (Newtons)	↑↑	↑↑	**Synergism**	$E = E_0 + E_{max} \times \frac{\left(\frac{C_eDet}{EC_{50}Det} + \frac{C_eMet}{EC_{50}Met} + \alpha \times \frac{C_eDet}{EC_{50}Det} \times \frac{C_eMet}{EC_{50}Met}\right)^{nDet,Met}}{1 + \left(\frac{C_eDet}{EC_{50}Det} + \frac{C_eMet}{EC_{50}Met} + \alpha \times \frac{C_eDet}{EC_{50}Det} \times \frac{C_eMet}{EC_{50}Met}\right)^{nDet,Met}}$	α = + 0.78
TT (°Celsius)	↑↑	=	**Positive potentiation of detomidine by methadone**	$E = E_0 + E_{max} \left[1 + p \times log\,(1 + C_eMet) \times \frac{C_eDet^n}{EC_{50}Det^n + C_eDet^n}\right]$	*pot* = + 0.133
ET (Volts)	↑↑	=	**Positive potentiation of detomidine by methadone**	$E = E_0 + E_{max} \left[1 + p \times C_eMet \times \frac{C_eDet^n}{EC_{50}Det^n + C_eDet^n}\right]$	*pot* = +0.0041
GIM (0 – 20 score)	↓↓	↓↓	**Infra-additive (antagonism)**	$E = E_0 \times \left[1 - I_{max} \times \frac{\left(\frac{C_eDet}{IC_{50}Det} + \frac{C_eMet}{IC_{50}Met} + \alpha \times \frac{C_eDet}{IC_{50}Det} \times \frac{C_eMet}{IC_{50}Met}\right)^{nDet,Met}}{1 + \left(\frac{C_eDet}{IC_{50}Det} + \frac{C_eMet}{IC_{50}Met} + \alpha \times \frac{C_eDet}{IC_{50}Det} \times \frac{C_eMet}{IC_{50}Met}\right)^{nDet,Met}}\right]$	α = - 1.05

Table 4: Summarised pharmacodynamic results of the effects of detomidine and methadone in 8 standing horses following 5 treatments administered intravenously: 0.2 mg/kg bwt methadone (MET), 5 µg/kg bwt detomidine alone (DET), or 0.2 mg/kg bwt methadone combined with 10 (MHD), 5 (MMD) or 2.5 (MLD) µg/kg bwt detomidine.