TITLE: A possible solution to model nonlinearity in elimination and distributional clearances with α2-adrenergic receptor agonists: Example of the intravenous detomidine and methadone combination in sedated horses

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Abstract

The alpha(α)$_2$-agonist detomidine is used for equine sedation with opioids such as methadone. We retrieved the data from two randomised, cross-over studies where methadone and detomidine were given intravenously alone or combined as *boli* (STUDY 1) (Gozalo-Marcilla et al., 2017) or as a 2-hr constant rate infusions (STUDY 2) (Gozalo-Marcilla et al., 2019a). Plasma drug concentrations were measured with a validated tandem Mass Spectrometry assay. We used Non-Linear Mixed Effect Modeling and took PK data from both studies to fit simultaneously both drugs and explore their non-linear kinetics. Two significant improvements over the classical mammillary two-compartment model were identified. First, the inclusion of an effect of detomidine plasma concentration on the elimination clearances of both drugs improved the fit of detomidine [Objective Function Value (OFV): -160] and methadone (OFV: -132) submodels. Second, a detomidine concentration-dependent reduction of distributional clearances of each drug further improved detomidine (OFV: -60) and methadone (OFV: -52) submodel fits. Using the PK data from both studies i) helped exploring hypotheses on the non-linearity of the elimination and distributional clearances, ii) allowed inclusion of dynamic effects of detomidine plasma concentration in the model which are compatible with the pharmacology of detomidine (vasoconstriction and reduction in cardiac output).

Keywords: Alpha(α)$_2$-adrenergic receptor agonist, cardiac output, equine, opioid, Non-Linear Mixed Effect Modeling, Pharmacokientics

Short communication

Alpha(α)$_2$-agonists and opioids such as detomidine and methadone are commonly combined in equine standing surgery to provide sedation and analgesia. Methadone and detomidine have been administered intravenously (i.v.) as a bolus for short-term procedures (Gozalo-Marcilla et al., 2017) or as constant rate infusions (CRIs) for prolonged surgeries (Gozalo-Marcilla et al., 2019a,b).

In a previous pharmacokinetic (PK) analysis after bolus administration (STUDY 1), there was evidence of interaction between the two drugs as fitting standard mammillary multi-compartment (Fig 1a) was unsatisfactory (Gozalo-Marcilla et al., 2018c). Indeed, non-linear PK was considered, as for different detomidine doses there was a non-proportional change in

the detomidine and methadone concentrations. Non-linearity can be explained by dose-dependent alteration of drug excretion or saturation of drug metabolism (Gabrielsson et al., 2016). In STUDY 1, the model including non-linearity on the elimination clearance (Cl) (Fig 1b) still over-predicted detomidine concentrations when administering low doses and under-predicted detomidine concentrations when administering high doses (Gozalo-Marcilla et al., 2019c). In a subsequent PK/PD study with the same group of horses (STUDY 2) (Gozalo-Marcilla et al., 2019a), the pharmacodynamics (PD) of methadone/detomidine combinations as CRIs were reported, but not the PK data. Non-linearity was also suspected as the ratio $\frac{Area\ Under\ the\ Curve\ (AUC)}{dose}$ was 30% higher with the high detomidine dose compared to the low dose. We hypothesised that combining the two PK datasets (STUDIES 1 & 2) would enable a better characterisation of the non-linearity on the clearance and the distribution associated with different detomidine doses. This report proposes a Michaelis-Menten equation solution to improve the fit of the observed non-linear elimination of both drugs.

We used data previously collected from 8 healthy adult horses (4 males, 4 females) receiving detomidine/methadone combinations (Table 1), in two crossover studies within a 2-year period. In STUDY 1, each horse received an i.v. bolus of detomidine alone, methadone alone or different combinations of both drugs. In STUDY 2, each horse received each of the following 4 treatments: i.v. bolus followed by a 2-hr CRI of detomidine (high and low dose), with detomidine alone or combined with a 2-hr CRI of methadone. For both studies, at least one week's washout period was allowed between treatments.

Venous blood was sampled from one designated jugular vein at predetermined time-points, between 0 and up to 360 minutes after treatment administration. Plasma detomidine and methadone were measured with a single and validated analytical method comprised of a liquid-liquid extraction technique with ethyl acetate for sample preparation and analysis by tandem Liquid Chromatography/Mass Spectrometry (Gozalo-Marcilla et al., 2019c).

Our working hypothesis was that one can model the plasma concentration-time profile of both drugs by including a specific relationship between PK parameters (Cl and inter-compartmental Cl) and concentration. The same non-linear mixed effect approach to PK modelling reported by Gozalo-Marcilla et al. (2019c) was used to analyse jointly the pooled plasma concentration time-curves of the two studies. We modelled the PK for both drugs together using a sequential approach; we solved the PK of detomidine first, then fixed parameter estimates (theta) and, if applicable, individual deviations (etas) before solving

methadone PK employing the same principles. A proportional error model was used. Inter-individual variability was estimated when enough information was available and eta shrinkage was kept to an acceptable level. Rival population PK models were designed to best fit the data and their performances were compared with Phoenix NLME 8.0 for visual inspection (goodness of fit plots) and statistically significant reduction in Objective Function Values (OFV).

Dose-dependent Cls (Fig 1b) were written according to equations 1a and 1b, respectively (as in Gozalo-Marcilla et al., 2019c):

$$Cl_{detomidine(c)} = Cl_{detomidine\_basal} \times (1 - S \times (\log(1 + [Detomidine])) \quad \text{Eq 1a}$$

$$Cl_{methadone(c)} = Cl_{methadone\_basal} \times (1 - P \times [Detomidine]) \quad \text{Eq 1b}$$

where S is the coefficient of moderation of detomidine Cl by detomidine plasma concentration [Detomidine]; p, coefficient of moderation of methadone Cl by [Detomidine].

This replicated well the results from Gozalo-Marcilla et al. (2019c) with increased parameter precision. One consequence was that methadone better fitted within a 2-compartment model instead of 3. Table 2 summarises the model improvement associated with inclusions of the two sources of non-linearity. Inclusion of a modulatory effect of detomidine on its own Cl and on methadone's Cl (Fig 1b & Step 2 in Table 2) improved detomidine's (OFV:1130 to 970) and methadone's (OFV: 6727 to 6595) submodels fittings. Two problems remained with this model: i) the apparent increase in plasma concentrations of both drugs in all horses at the end of the infusion could not be fitted (concentrations consistently under-estimated) and ii) consistent over-estimation of detomidine plasma concentrations after the smaller doses of detomidine.

In this manuscript, we hypothesised a concentration-dependent effect of [Detomidine] on the distribution on both drugs (distributional clearance Cl_2). [Detomidine] reduced unidirectionally the inter-compartment transfer rate constant from central to peripheral compartment, using an adaptation of a Michaelis-Menten model (equation 2), whereas the transfer rate constant from peripheral to central compartment did not change as a function of [Detomidine] (equation 2) (Fig 1c).

The transfer rate constant from central to peripheral compartments for detomidine ($CL_{2\ c\rightarrow p}$) and methadone ($CL_{2m\ c\rightarrow p}$) was written as in equation 2a:

$$Cl_{2\,c\to p} = {V_{max}}/{(K_m + [Detomidine])} \text{ and } Cl_{2m\,c\to p} = {V_{maxm}}/{(K_{mm} + [Detomidine])}$$

Eq 2a

whereas the transfer rate constant from peripheral to central compartment for detomidine ($CL_{2\,p\to c}$) and methadone ($CL_{2m\,p\to c}$) remained as in equation 2b:

$$Cl_{2\,p\to c} = {V_{max}}/{(K_m)} \quad \text{and} \quad Cl_{2m\,p\to c} = {V_{maxm}}/{(K_{mm})} \quad \text{Eq 2b}$$

V_{max} and V_{maxm} were the maximal transfer speed for detomidine and methadone respectively (expressed in $\mu g\ kg^{-1}\ hr^{-1}$); K_m and K_{mm} were the concentration of detomidine or methadone at half of V_{max}.

Inclusion of a dose-dependent effect on distributional Cl as represented in Fig 1c (Step 3 in Table 2) improved model fit and OFVs for detomidine (OFV: 970 to 910) and methadone submodels (OFV: 6595 to 6543)]. Final parameters of this model are summarised in Table 3. Individual fits are presented in Figure 2. Other solutions were explored and this specific one was the most satisfactory at the time of manuscript write-up, but one cannot exclude that a better solution could exist.

In horses, α_2-adrenergic receptor agonists, such as detomidine, increase systemic vascular resistance (SVR) and decreases heart rate (HR), therefore decreasing cardiac output (CO) (Yamashita et al., 2000); for drugs whose elimination are flow-dependent, Cl depends on liver perfusion and CO. Only few studies include concentration-dependent cardiovascular effects induced by a given drug in its PK model. Cardiac output influences distribution kinetics of alfentanil in conscious humans (Henthorn et al., 1992) and halothane-anaesthetized pigs (Kuipers et al., 1999); alfentanil's depressant effects on CO can be counteracted by the analeptic doxapram, as increases distribution and elimination Cls of alfentanil (Roozekrans et al., 2017). Dutta et al. (2000) used HR as a surrogate for CO to improve the fit of dexmedetomidine PK in man, without relating dexmedetomidine concentrations directly to CO though. When dexmedetomidine was infused to isoflurane-anaesthetised cats, CO measurements obtained at steady state and included in a modified 2-compartment model helped modelling the effect of plasma dexmedetomidine concentrations on its own Cl (Pypendop et al., 2013). This was supported by the restoration of medetomidine's Cl when administered with atipamezole in dogs (Salonen et al., 1995).

Our research did not focus on concomitant CO changes. However, the knowledge of detomidine concentration-time profiles allowed comparison with other equine studies that reported concomitantly plasma concentrations and CO. Detomidine i.v. *boli* produced transitory vasoconstriction increasing SVR and arterial blood pressures, decreasing HR and CO (Yamashita et al., 2000); similar effects occurred when infused at four different target-concentration rates (Daunt et al., 1993). In our CRI study (Fig 2, STUDY 2), the detomidine plateau concentrations increased from 2 to 3 µg/L for the low dose (2.5 µg/kg + 12.5 µg kg^{-1} hr^{-1} over 2 hr, treatments F and H) and from 7 to 9 µg/L for the high dose (5 µg/kg + 25 µg kg^{-1} hr^{-1} over 2 hr, treatments G and I). These concentrations are roughly in line with the two lowest target concentrations from Daunt et al. (1993) (infusion 1 and 2, respectively), demonstrating a concentration dependent effect of detomidine alone on CO (for all target plasma concentrations) and SVR (at higher target plasma concentrations).

From our *in vivo* PK/PD modelling, data analysis showed non-linearity for both drugs at different levels of detomidine concentrations (Gozalo-Marcilla et al., 2019c). First, the empirical PK model included an effect of detomidine on the Cl of both drugs (Fig 1b), compatible with the observed effect at all plasma concentrations (Daunt et al., 1993). Second, the empirical model reduced the distribution of both drugs to the peripheral compartment at high plasma detomidine concentrations (Fig 1c), consistent with the vasoconstrictive effects of detomidine at higher plasma concentrations (Daunt et al., 1993). Detomidine-induced peripheral vasoconstriction is mediated via subtype receptors α_{2b} in vascular smooth muscle (Link et al., 1996). As vasoconstriction occurs, there is a possibility that the "shrinking" peripheral distribution increases central organ perfusion, reducing the volume of distribution of other drugs (Bennett et al., 2017). These effects may be reduced by co-administering the antagonist MK-467, as reported in cats (Honkavaara et al., 2017; Pypendop et al., 2017) and horses (Pakkanen et al., 2015; de Vries et al., 2016).

Some alternative strategies to model non-linearity were explored. Including a time dose-dependency in the expression of the volume of distribution is not allowed by basic function of Phoenix (due to circular referencing as concentrations are defined by amounts/volumes within a given compartment) but it is not impossible and could be programmed. The strategy from Roozerkans et al. (2017) to include the doxapram-induced increase in cardiac output in the expression of elimination and distributional clearances of alfentanil could not be tested in our setting but supports the concept that α_2-adrenergic receptor agonists could affect elimination and distributional clearances in a PK model. While we

present a satisfactory solution to non-linearity, there may be better solutions that improve either the fitting of the data or the numerical stability of the model. Including an effect-site concentration of detomidine that could limit elimination and distribution clearances of both drugs through a pharmacodynamic function (Imax model for example) would be worth exploring (Roozerkans et al. 2017).

To conclude, pooling the PK data from both studies i) helped confirming the non-linearity of the elimination Cl and ii) allowed to explore hypotheses on the non-linearity of the distributional clearance (Michaelis-Menten equation) that could not be explored with only the first PK dataset (STUDY 1).

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