Effects of vatinoxan on cardiorespiratory function and gastrointestinal motility during constant-rate medetomidine infusion in standing horses

H. TAPIO*, M. R. RAEKALLIO†, A. MYKKÄNEN†, S. MÄNNIKÖ†, M. SCHEININ§, R. C. BENNETT† and O. VAINIO‡

†Faculty of Veterinary Medicine, Department of Equine and Small Animal Medicine, University of Helsinki, Helsinki, Finland
‡4Pharma Ltd., Turku, Finland
§Institute of Biomedicine, University of Turku, Turku, Finland
Faculty of Veterinary Medicine, Department of Equine and Small Animal Medicine, University of Helsinki, Helsinki, Finland

*Correspondence email: heidi.tapio@helsinki.fi; Received: 21.06.18; Accepted: 17.02.19

Summary

Background: Medetomidine suppresses cardiovascular function and reduces gastrointestinal motility in horses mainly through peripheral α2-adrenoceptors. Vatinoxan, a peripheral α2-antagonist, has been shown experimentally to alleviate the adverse effects of some α2-agonists in horses. However, vatinoxan has not been investigated during constant-rate infusion (CRI) of medetomidine in standing horses.

Objectives: To evaluate effects of vatinoxan on cardiovascular function, gastrointestinal motility and on sedation level during CRI of medetomidine.

Study design: Experimental, randomised, blinded, cross-over study.

Methods: Six healthy horses were given medetomidine hydrochloride, 7 µg/kg i.v., without (MED) and with (MED+V) vatinoxan hydrochloride, 140 µg/kg i.v., followed by CRI of medetomidine at 3.5 µg/kg/h for 60 min. Cardiorespiratory variables were recorded and borborygmi and sedation levels were scored for 120 min. Plasma drug concentrations were measured. The data were analysed using repeated measures ANCOVA and paired t-tests as appropriate.

Results: Initially heart rate (HR) was significantly lower and mean arterial blood pressure (MAP) significantly higher with MED compared with MED+V. For example at 10 min HR (mean ± s.d.) was 26 ± 2 and 31 ± 5 beats/minute (P = 0.04) and MAP 129 ± 15 and 103 ± 13 mmHg (P < 0.001) respectively. At 10 min, cardiac index was lower (P = 0.02) and systemic vascular resistance higher (P = 0.001) with MED than with MED+V. Borborygmi were reduced after MED; this effect was attenuated by vatinoxan (P < 0.001). All horses were sedated with medetomidine, but the mean sedation scores were reduced with MED+V until 20 min (6.8 ± 0.8 and 4.5 ± 1.5 with MED and MED+V, respectively, at 10 min, P = 0.001). Plasma concentration of dexmedetomidine was significantly lower in the presence of vatinoxan (P = 0.01).

Main limitations: Experimental study with healthy, unstimulated animals.

Conclusions: Vatinoxan administered i.v. with a loading dose of medetomidine improved cardiovascular function and gastrointestinal motility during medetomidine CRI in healthy horses. Sedation was slightly yet significantly reduced during the first 20 min.

The Summary is available in Portuguese – see Supporting Information

Keywords: horse; sedation; medetomidine; vatinoxan; MK-467; α2-antagonist

Introduction

The α2-adrenoceptor agonists (α2-agonists) administered by constant-rate infusions (CRI) are commonly used to provide sedation for standing surgical and diagnostic procedures. Medetomidine, with its pharmacologically active enantiomer dexmedetomidine, is the most selective α2-agonist in clinical use and provides sedation and analgesia through centrally mediated effects [1]. In horses, its pharmacokinetic profile, with a short half-life, makes it attractive for use in CRI [2,3], although it is registered for use only in small animals. Infusion rates for stable sedation with medetomidine and dexmedetomidine in horses have been described [2-4]. However, medetomidine shares the various adverse peripheral effects of all α2-agonists. Most notably, medetomidine causes vasoconstriction, leading initially to hypertension and baroreceptor-mediated reflex bradycardia [5], later followed by a centrally mediated hypotension [4]. At equipotent doses, medetomidine-induced bradycardia is shorter in duration than that induced by detomidine [6]. Additionally, medetomidine decreases mixed venous oxygen tension [4]. Dexmedetomidine also decreases borborygmi and increases blood glucose concentrations in horses [7].

Vatinoxan (previously MK-467 or L-659,066) is a peripherally acting α2-adrenoceptor antagonist. In rats and marmosets, vatinoxan crosses the blood-brain barrier minimally such that its concentration in the brain remains far below that present in blood [8]. Thus, vatinoxan may reduce the peripheral adverse effects of α2-agonists without interfering with their centrally mediated sedative or analgesic effects. In previous equine studies, vatinoxan prevented the adverse cardiovascular effects of simultaneously administered single doses of the α2-agonists medetomidine [9], detomidine [10] and romifidine [11]. Although vatinoxan increased the volume of distribution and clearance of detomidine [10] and romifidine [11] in horses, it did not have clinically relevant effects on the degree of sedation. Furthermore, vatinoxan improved gastrointestinal motility in horses when administered with single doses of detomidine [10,12] and romifidine [11].

The objective of this study was to evaluate the impact of vatinoxan on cardiorespiratory and gastrointestinal parameters during a CRI of medetomidine in standing, healthy horses. In addition, the effects of vatinoxan on sedation and plasma concentrations of the enantiomers of medetomidine (dexmedetomidine and levomedetomidine) were investigated. We hypothesised that vatinoxan would alleviate the cardiovascular
and gastrointestinal depression caused by medetomidine but would not lead to clinically relevant loss of sedation.

**Materials and methods**

**Horses**

Six horses (4 Standardbreds and 2 Warmbloods; 3 mares and 3 geldings) with a median age of 10 years (range, 5–19 years) and median body weight of 513 kg (range, 460–557 kg) were used in the study. The horses were considered healthy on the basis of clinical examination and results of complete blood count and serum biochemistry. Horses were fed hay and had free access to water, except during the experiment. Feed, but not water, was withheld for 2 h before and after the experiment.

**Instrumentation**

Instrumentation was performed with horses unmedicated and restrained in stocks. First, vascular catheters were aseptically placed with 2 mL of 2% mepivacaine (Scandicain) injected subcutaneously for local anaesthesia. A central venous catheter (Cavafix Certo) was placed into the cranial vena cava via the distal part of the left jugular vein. The correct location of the central venous catheter was verified by characteristic pressure wave forms on the monitor screen (S/5 compact Critical Care Monitor). The proximal part of the left jugular vein was then used for a standard intravenous catheter (Intraflox). An arterial catheter (BD Arterial cannula) was placed into the previously subcutaneously relocated [13] right common carotid artery. Finally, a lithium sensor (LiDCO Plus) was connected to the arterial catheter for cardiac output (Qt) determination by the lithium dilution method (LiDCO Plus) [14]. Lithium was prepared as previously described by adding 1.875 mL of 8 mol/L lithium chloride to 100 mL of saline (0.9%) solution to obtain a concentration of 150 mmol/L [12]. Standard values of arterial and venous blood pressure, arterial and venous oxygen content, arterial and venous oxygen saturation, arterial and venous oxygen difference, and haemoglobin concentration were determined. Heart rate and respiratory rate were obtained by auscultation, and Qt, systolic (SAP), mean (MAP) and diastolic (DAP) arterial blood pressure were recorded. Arterial and venous blood samples were collected into EDTA-containing tubes at 10, 30, 60 and 120 min and stored at room temperature (20°C). Plasma was separated by centrifugation at 400 g for 10 min at 26 °C after sample collection and stored at −20°C until analysed for drug concentrations.

**Treatments and data collection**

Prior to drug administration, baseline values for all measurements were determined. Heart rate and respiratory rate were obtained by auscultation, and Qt, systolic (SAP), mean (MAP) and diastolic (DAP) arterial blood pressure and central venous pressure were recorded. Arterial and venous blood samples were collected into EDTA-containing tubes and stored at −20°C until analysis for blood gases.

**Analysis of plasma drug concentrations**

The concentrations of drugs in plasma were determined with high-performance liquid chromatography-tandem mass spectrometry in separate aliquots that were processed under specific conditions for the two drugs. The concentrations were determined after solid-phase extraction with well extraction plates (Sep-Pak TC18) with racemic d₃-medetomidine (Toronto Research Chemicals) and RS-79948 (Tocris Biosciences) as the respective internal standards. For dexmedetomidine and levomedetomidine analysis, chiral separation with an α,α-dicyclohexylpropyl (AGP) column (Chiralpak) was performed and 10 mmol/L ammonium acetate (pH 4.5) and acetone/trimethylamine (0.1% formic acid) were used as solvents. For vatinoxan, reversed-phase separation with a 2.1 × 150 mm, 3.5 μm C₁₈ column (SunFire) and a gradient solvent system (0.1% formic acid in water and acetonitrile) was used. Quantitative detection was performed in multi-reaction monitoring mode with a triple quadrupole mass spectrometer (4000 QTrap). For dexmedetomidine and levomedetomidine and for d₃-medetomidine, the respective precursor ions (m/z) were 201.2 and 204.2. The fragment ions (m/z) monitored and used for quantitation were 95.1 for dextrmedetomidine and levomedetomidine and 98.05 for d₃-medetomidine. For vatinoxan and RS-79948, the respective precursor ions (m/z) scanned were 419.3 and 365.3. The fragment ions monitored and used for quantitation were 200.1 for vatinoxan and 190.2 for RS-79948. The chromatograms were then processed using MSD ScieX software. The linear concentration range for the assay of dexmedetomidine and levomedetomidine was from 0.100 to 10.0 ng/mL. The inter-assay accuracy of the quality control samples (at three different concentration levels, 70, 250 and 380 ng/mL) ranged from 102.5 to 113.6.

**Data analysis**

All statistical analyses were performed using commercially available software (SAS® and SPSS®). The sample size was chosen based on practical considerations and previous evidence from comparable studies [10,12] indicating that to detect clinically relevant differences in the investigated parameters a sample size of six horses would be sufficient.

Change from baseline was used as the response with all variables, excluding plasma drug concentration data. The differences between treatments in change from baseline values were assessed with repeated measures analysis of covariance models. The model consisted of a baseline covariate, the main effects of treatment, period, sequence and time point of measurement and two-way interactions of period*time point, sequence*time point and treatment*time point as fixed effects, and main effect of horse and two-way interaction terms of period*horse and time point*horse as random effects. No carry-over effect was expected to arise in the study and was therefore not tested. The normality assumptions were checked for all responses with Kolmogorov–Smirnov tests. For SVI, DO₂I and VO₂I, logarithmic transformation was computed to normalise the distributions. For SAP, a square transformation and for CI an inverse transformation was used. With all transformed change variables, the model was fitted for changes in the transformed response. From the fitted models, estimates of treatment effect were calculated over time and by time point using model

Effects of vatinoxan during medetomidine infusion in standing horses

H. Tapio et al.

contrasts. In addition, estimates for the within treatment changes were computed similarly from the same model.

For plasma concentration values and areas under the time-plasma concentration curves, normality was investigated with the Shapiro-Wilk test. The data were normally distributed and paired t-tests were applied. P≤0.05 were considered statistically significant.

Materials and methods

Fig 1: Mean (±s.d.) heart rate of 6 horses after administration of medetomidine hydrochloride 7 µg/kg i.v. alone (MED) or with vatinoxan hydrochloride 140 µg/kg i.v. (MED+V) at 0 min and constant-rate infusion of medetomidine 3.5 µg/kg/h administered from 5 to 65 min. Significant differences (P<0.05) are labelled as within (a) MED and (b) MED+V with respect to baseline and as (*) between the treatments at given time points.

Fig 2: Mean (±s.d.) arterial blood pressure of 6 horses after administration of medetomidine hydrochloride 7 µg/kg i.v. alone (MED) or with vatinoxan hydrochloride 140 µg/kg i.v. (MED+V) at 0 min and constant-rate infusion of medetomidine 3.5 µg/kg/h administered from 5 to 65 min. Significant differences (P<0.05) are labelled as within (a) MED and (b) MED+V with respect to baseline and as (*) between the treatments at given time points.

Fig 3: Mean (±s.d.) systemic vascular resistance index of 6 horses after administration of medetomidine hydrochloride 7 µg/kg i.v. alone (MED) or with vatinoxan hydrochloride 140 µg/kg i.v. (MED+V) at 0 min and constant-rate infusion of medetomidine 3.5 µg/kg/h administered from 5 to 65 min. Significant differences (P<0.05) are labelled as within (a) MED with respect to baseline and as (*) between the treatments at given time points.

Results

Cardiovascular effects

Heart rate was overall significantly lower with MED than with MED+V; the lowest HR was 24 ± 3 beats/minute and 30 ± 3 beats/minute with MED and MED+V, respectively (Fig 1). With MED+V, MAP immediately decreased and was significantly lower than with MED until 60 min, with a nadir of 100 ± 19 mmHg at 30 min (Fig 2). MAP remained unchanged from baseline with MED until 45 min, after which MAP significantly decreased, with a nadir of 97 ± 10 mmHg at 120 min (Fig 2). Similar changes were observed in SAP and DAP (Table 1). A significant increase in CVP was observed with MED until 20 min, after which CVP remained at baseline levels, whereas a significant decrease in CVP was observed with MED+V at 30 min, lasting until 90 min.

With MED, the CI decreased and SVRI increased immediately and were significantly different from MED+V at 10 min (Table 1 and Fig 3). After cessation of the CRI, there was a significant decrease in SVRI and increase in SVI with MED (Table 1 and Fig 3). In comparison, no significant changes in CI, SVRI and SVI were detected with MED+V. Left ventricular workload significantly decreased after medetomidine administration, without significant differences between the treatments (Table 1).

Respiratory rate and arterial and venous blood gas analysis

Respiratory rate, PaO2, Pco2 and DO2i significantly decreased, and O2ER and CO2 tensions in arterial and venous blood significantly increased over time with both treatments [data for 5 and 45 min and for CO2 tension are not shown] (Table 2). However, significant differences were detected only in Pvo2 and in O2ER, in which the changes from baseline were significantly greater with MED than with MED+V. No consistent changes in VO2i were detected (Table 2). Arterial glucose concentrations did not change from baseline with MED+V whereas they increased significantly with MED, with the highest mean value of 9.6 ± 2.4 mmol/L (Table 2).

Borborygmi and sedation scores

While medetomidine significantly decreased the borborygmi score, the decrease was overall significantly smaller and briefer with MED+V than with MED (Table 1). The sedation score increased significantly from baseline in all horses within 10 min after administration of medetomidine; scores were increased from 0.7 ± 0.5 to 6.8 ± 0.8 and from 0.5 ± 0.6 to 4.5 ± 1.5 with MED and MED+V respectively. Sedation scores returned to
The results of this study support the hypothesis that a single i.v. bolus of vatinoxan prevents the cardiovascular and gastrointestinal effects induced by a loading dose and CRI of medetomidine without major effects on pre-treatment levels by 120 min after drug administration (Table 1). The increase in sedation score was significantly larger with MED than with MED+V at 10 and 20 min.

**Pharmacokinetic results**

The areas under the time-plasma concentration curves of dexmedetomidine and levomedetomidine were significantly smaller in the presence of vatinoxan, thus indicating lower plasma exposure to dexmedetomidine (Table 3, Fig 4). Indeed, the concentrations of dexmedetomidine in plasma during the CRI of medetomidine were also significantly lower with MED+V than with MED (Fig 4).

**Discussion**

The results of this study support the hypothesis that a single i.v. bolus of vatinoxan prevents the cardiovascular and gastrointestinal effects induced by a loading dose and CRI of medetomidine without major effects on...
sedation. As expected, medetomidine, when administered alone, induced immediate vasoconstriction (observed as increased MAP and SVRI), even if MAP remained within clinically acceptable limits. From 60 min onwards MAP and SVRI decreased with MED, likely due to prevailing central sympatholytic effect of medetomidine [4]. In the presence of vatinoxan, MAP decreased but remained within the reference range of standing horses (94–140 mmHg) [20]. Furthermore, the calculated SVRI (describing peripheral vasoconstriction) remained stable with MED+V throughout the study period. The observations likely reflect the medetomidine-induced vasoconstriction that vatinoxan alleviated, probably via its direct antagonism of vascular $\alpha_2$-adrenoceptors. No bradycardia was observed with MED+V, likely due to lack of baroreceptor responses in the context of normal systemic vascular resistance [5]. The observed fall in heart rate with MED+V can likely be attributed to reduced sympathetic tone. These findings are consistent with previous studies on horses, in which vatinoxan was administered simultaneously with single bolus doses of medetomidine [9], detomidine [10] or romifidine [11]. Noticeably, the baseline MAP readings in this study were unexpectedly high, which may reflect a stress response of these unsedated horses, standing in stocks prior to the start of the experiment. Similarly, stress and consequent moving of horses could at least partially explain the relatively large variation in the CI measurements at 120 min. The arterial catheters functioned normally and LiDCO system at 120 min should be treated with caution.

Decreased Qt is a recognised effect of medetomidine in horses [4,6,22]. In dogs, dexmedetomidine did not have a direct myocardial depressant effect; the decrease in cardiac output and stroke volume were likely consequences of its other haemodynamic effects [23]. Thus, it is plausible that as SVR remained stable and bradycardia did not ensue with MED+V, CI also remained unchanged. The increased CVP initially seen after medetomidine and its attenuation by vatinoxan are in line with the other haemodynamic findings of this study. Increased CVP suggests increased preload, which has been attributed to peripheral and pulmonary vasoconstriction caused by $\alpha_2$-agonists [6]. Interestingly, some evidence also suggests that equine saphenous veins may express adrenergic receptors [24], in which case medetomidine and vatinoxan may additionally have direct effects on venous walls. Our CVP observations agree with a previous study that combined vatinoxan with an i.v. bolus dose of detomidine in horses [10]. In contrast, increased CVP has not been consistently observed after medetomidine [4]. This could be partially explained by the use of a smaller dose of medetomidine than in our study, as the cardiovascular effects of medetomidine seem to be dose-dependent within a certain range [6]. Finally, the cardiovascular suppression in horses during CRI of medetomidine has been reported to be minor after the changes following the medetomidine loading dose have waned [2]. Although there was also a tendency in this study for most of these haemodynamic changes to occur early after the medetomidine loading dose, these changes could be alleviated by vatinoxan.

In this study, vatinoxan had no detectable effect on the observed transient (although minor) decrease in PaO2 after medetomidine. Nevertheless, small but significant decreases in PvO2 after medetomidine were observed, and were found to be alleviated by vatinoxan, similarly to previous reports [4,22]. Furthermore, vatinoxan improved the O2ER, which describes the amount of oxygen removed from blood by tissues. The
oxygent extraction ratio increases in situations such as tissue hypoxia and increased metabolic oxygen demand [25]. Thus, improved O2ER probably reflects better tissue perfusion in horses receiving vatinaxan. The improved VO2 and O2ER with vatinaxan paralleled the improved haemodynamic parameters (i.e. SVRI and CI) during the CRI, as presumed. The improved oxygenation and tissue perfusion may become clinically important in sedation protocols for surgical procedures or in horses that have a systemic illness and compromised cardiovascular function, such as horses with severe colic.

α2-agonists are known to increase plasma glucose concentrations by decreasing insulin secretion via activation of α2-adrenoceptors located on pancreatic β-cells, and this was observed in this study as well with medetomidine, as also previously reported [26]. Also, this effect was alleviated by vatinaxan, probably via blockade of pancreatic α2-adrenoceptors, as seen in dogs [27].

In this study, medetomidine decreased borborygmi (as assessed by auscultation) as has been reported previously [26]. α2-adrenoceptors with an inhibitory effect on motility are expressed in equine jejunum [28]. In addition, the equine caecum and colon are postulated to be more sensitive to the effects of medetomidine than the jejunum [29], which is supported by a previous observation of decreased faecal output after medetomidine for 3.5–5 h [26]. This increases the risk for post-sedation colon impaction and colic. In our study, vatinaxan alleviated the decrease in gastrointestinal borborygmi scores throughout the observation period, and the scores were even restored to baseline levels sooner in the presence of vatinaxan. This is consistent with previous studies where vatinaxan prevented [10] and reversed [12] gastrointestinal hypomotility after administration of detomidine. Direct antagonism of vatinaxan at the level of intestinal α2-adrenoceptors could explain the improved gastrointestinal motility. Thus, vatinaxan may reduce the risk of post-sedation colic in horses, especially after more prolonged sedation with a CRI of medetomidine.

All horses were sedated after administration of medetomidine. Vatinaxan slightly yet significantly decreased the mean sedation scores during the first 20 min. The decreased sedation scores may at least in part be explained by the differences in the plasma concentrations of dexmedetomidine between the two treatments. Vatinaxan has previously been reported to enhance the distribution and clearance of i.v. bolus doses of detomidine [10] and romifidine [11] in horses, most likely through changes in haemodynamics. In the aforementioned studies, vatinaxan decreased the mean sedation scores [11] or areas under the time-sedation score curves, albeit the maximum sedation scores were unaffected [10]. Furthermore, in dogs the distribution of an i.v. bolus dose of dexmedetomidine was increased by concomitant vatinaxan mainly during the first 10 min, after which the elimination curves of dexmedetomidine with and without vatinaxan appeared parallel [30].

Although pharmacokinetics were not investigated in such detail in this study, the sedation scores differed between the treatments only in the first 20 min following the administration of vatinaxan and the loading dose of medetomidine. This supports the concept of vatinaxan promoting the early distribution of dexmedetomidine. In horses, the half-lives of medetomidine and dexmedetomidine are short (11–29 min [2,26] and 19–29 min [3,22] respectively). Whilst, the pharmacokinetic steady state of medetomidine is attained within approximately 30 min using a CRI and a smaller loading dose (5 μg/kg) than that used in this study [2]. In comparison, the half-life of vatinaxan in horses has been reported to be markedly longer than that of (dex)medetomidine; over 2 h when given alone [11]. In this study, vatinaxan half-life was approximately 75 min (mean) in the presence of medetomidine. Thus, it is plausible that using a medetomidine CRI, steady state was achieved in our horses, and, furthermore, that the antagonistic effect of the single dose of vatinaxan persisted during this phase without major impact on the sedation level.

A limitation of this study was the use of healthy, unstimulated animals. The effects of vatinaxan on sedation quality and on the potential analgesic effect of medetomidine when a nociceptive stimulus is applied, remains to be investigated.

In conclusion, vatinaxan administered simultaneously with a loading dose of medetomidine improved cardiovascular and gastrointestinal function during a CRI of medetomidine in healthy horses. This was achieved without noticeable side effects or clinically relevant effects on sedation scores. By improving the haemodynamics of horses during sedation with medetomidine, vatinaxan could also improve tissue perfusion during sedation. Improved gastrointestinal motility may reduce the risk of post-sedation colic. The safety of vatinaxan in clinically ill horses and its effects on the analgesic efficacy of α2-agonists warrant further studies.

Authors’ declaration of interests

No competing interests have been declared.

Ethical animal research

This study was approved by the National Animal Experiment Board of Finland (No. ESAVI/4789/04.10.07/2014).

Owner informed consent

Not applicable.

Sources of funding

Financial support was provided by Vetcare Ltd. (Mäntsälä, Finland) for the study expenses; materials and drugs.

Acknowledgements

The authors thank Prof Paula Larenza for her contribution to the study design.

Authorship

H. Tapio and M. Raekallio contributed to study design, study execution, data analysis and interpretation and preparation of the manuscript. A. Mykkänen contributed to study design, study execution and preparation of the manuscript. S. Männikkö and M. Scheinin contributed to data analysis and interpretation, and preparation of the manuscript. R. Bennett contributed to study execution and preparation of the manuscript. O. Vainio contributed to study design and preparation of the manuscript. All authors gave their final approval of the manuscript.

References

Effects of vatinoxan during medetomidine infusion in standing horses

H. Tapio et al.


Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Summary in Portuguese.

Supplementary Item 1: Sedation scores.

Supplementary Item 2: Borborygmi scores.

Supplementary Item 3: Calculated indices.