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Bronchodilator activity of the selective muscarinic antagonist revatropate in horses with heaves

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ABSTRACT

Bronchodilators are frequently used to attenuate airway obstruction in equine heaves (or recurrent airway obstruction). This study evaluated the selective (M_3 and M_1) muscarinic antagonist revatropate, which offers potential advantages over non-specific antimuscarinic agents such as ipratropium. Protocol 1 assessed the response to inhaled revatropate (1, 2 and 7 mg) using a blinded, negative (inhaled saline) and positive (inhaled ipratropium bromide; 0.3, 0.7 and 2 mg) controlled, dose escalation study, with six heaves horses. The lowest doses of revatropate and ipratropium induced a rapid (within 1 h) and significant improvement in airway function. The highest doses of both drugs had no significant effect on gastrointestinal sound score or iris function, but resulted in tacky mucous membranes and reduced gastrointestinal sound score in some horses.

In Protocol 2, a cross-over design comparing the duration of action of inhaled revatropate (1 mg), ipratropium (0.3 mg) and saline, some indices of airway function were improved for between 5 and 6 h after revatropate administration, and for between 6 and 24 h after ipratropium administration. Inhaled revatropate and ipratropium had similar effects on airway function, with no significant difference between their efficacies. Importantly, however, only revatropate significantly improved clinical scores of breathing effort, improving combined clinical score at the 1 h time point and abdominal score at the 1–3 h time points. No significant adverse events were observed in Protocol 2, although some horses had reduced gastrointestinal sound scores. Inhaled revatropate is therefore a safe and effective bronchodilator for treating airway obstruction in heaves.

Introduction

Heaves is characterised in horses by recurrent airway obstruction, partly due to airway smooth muscle contraction evoked by activation of muscarinic receptors. Consequently, inhalation therapy with muscarinic antagonists such as ipratropium has proven efficacy in attenuating airway obstruction in cases of heaves (Hoffman et al., 1993; Robinson et al., 1993; Duvivier et al., 1997). Revatropate offers potential advantages over non-specific antimuscarinic agents such as ipratropium, since it has significantly greater selectivity for the M_3 and M_1 muscarinic receptors on airway smooth muscle which mediate bronchoconstriction (Zaagsma et al., 1997) than for M_2 receptors on cardiac muscle, thereby reducing undesirable cardiac effects such as tachycardia (Alabaster, 1997). Furthermore, unlike ipratropium, revatropate does not block the presynaptic inhibitory M_2 receptors which may potentiate bronchoconstriction (Alabaster, 1997). The present study compared the efficacy and adverse effects of inhaled revatropate and ipratropium in heaves horses.

Materials and methods

Horses

Six horses with heaves were used (3 geldings, 3 mares; median age 15 years, range 7–>25 years; median weight 363 kg, range 253–561 kg). The diagnosis of heaves was confirmed as described previously (Pirie et al., 2001, 2002, 2003) and was consistent with an expert consensus statement (Robinson, 2001). Prior to commencing the study, horses had not received corticosteroids for ⩾3 months or any other drugs for ⩾2 weeks. Horses were trained to stand in stocks and undergo airway mechanics testing without restraint.

The study was approved by the University of Edinburgh Ethical Review Committee and by The Home Office, and was conducted under Home Office project licence PPL 60/2722.

Collection of data when horses were in disease remission

Clinical examination and airway mechanics testing were performed when horses were in disease remission, having been maintained in a minimum dust environment as previously described (Pirie et al., 2001). Data collected included respiratory rate (RR), combined clinical score of breathing effort which was the sum of

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abdominal and nostril scores (both graded 0–4) as previously described (Rush et al., 1998; Robinson et al., 2000; Camargo et al., 2007), maximum change in transpleural pressure (dPpl), total airway resistance (R_L), work of breathing (W_b), heart rate (HR), gastrointestinal sound score, iris function and oral mucous membrane moistness (moist, tacky or dry). Airway mechanics testing was performed as previously described (Pirie et al., 2001, 2002, 2003). Gastrointestinal sound score was the sum of the scores for all four quadrants (dorsal and ventral on the left and right sides), with each being scored as 0 (no or minimal sound), 1 (haustral sounds only) or 2 (haustral and peristaltic sounds). Iris function was determined by visual assessment of pupil size and of the pupillary light response.

Protocol 1: Dose escalation study

Protocol 1 was a three period cross-over, dose escalation study. Horses were exposed to mouldy hay/straw until they developed a combined clinical score of breathing effort $\geqslant 5$ and dPpl $\geqslant 1.96$ kPa (equivalent to 20 cm H_2O) ($T = 0$ h). Three incremental 1 mL volumes of revatropate (1, 2 and 7 mg), ipratropium bromide (0.3, 1 and 3 mg) or saline (placebo) were then administered by inhalation at 1 h intervals ($T = 0$, $T = 1$ and $T = 2$ h). Treatment order was randomized, and each treatment was separated by a wash out period of $\geqslant 48$ h. Aerosols were produced and delivered as previously described (Pirie et al., 2003). Clinical examination and airway mechanics testing were performed by a blinded observer between 45 and 60 min after each administration.

Protocol 2: Determining the duration of action of revatropate and ipratropium

Protocol 2, a three period cross-over design, was performed $\geqslant 1$ week after completion of protocol 1. Horses were exposed to mouldy hay/straw until they developed a combined clinical score of breathing effort $\geqslant 5$ and dPpl $\geqslant 1.96$ kPa (equivalent to 20 cm H_2O) ($T = 0$ h). Single optimal doses of inhaled revatropate and ipratropium as determined in protocol 1 (1 and 0.3 mg, respectively) and placebo (saline) were then administered in 1 mL volumes in randomised order, with $\geqslant 48$ h between treatments. The effects of treatments were assessed as described for protocol 1, but at hourly intervals up to 6 h and then at 24 h. Horses were maintained in the mouldy hay/straw environment during this period.

Statistical method

For Protocol 1 two sets of analyses were done. The first considered whether in remission data differed from those at $T = 0$, 1, 2 and 3 h for the three treatments (revatropate, ipratropium and saline) separately. The second compared data for $T = 0$ h with those for $T = 1$, 2 and 3 h, with again the three treatments being considered separately. For all variables, except abdomen, nostril, and combined clinical scores and GIT sounds, linear mixed effect models on $\log_{10}$ transformed variables (to normalise the residuals) were done. The horse was entered as the random effect to account for the repeated measures from the same horses being used in the three treatments. Time (in remission, $T = 0$, 1, 2, and 3 h for first set of analyses and $T = 0$ h, and $T = 1$, 2 and 3 h for the second set of analyses) was entered as the fixed effect. For the first set of analyses, in remission was entered as the reference level and for the second set of analyses $T = 0$ h. For the three clinical scores and GIT sounds a series of paired analyses using Wilcoxon signed rank tests were done, with the three treatments being considered separately. The paired comparisons were in remission data vs. $T = 0$, 1, 2 and 3 h for the first set of analyses and $T = 0$ h values vs. $T = 1$, 2 and 3 h for the second set of analyses.

For Protocol 2 a similar set of analyses were done with linear mixed effect models on $\log_{10}$ transformed data used for most of the parameters and Wilcoxon signed rank paired tests for the clinical scores and GIT sounds. For each treatment, in remission data were compared to $T = 0$, 1, 2, 3, 4, 5, 6 and 24 h data for the first set of analyses and $T = 0$ h data were compared with $T = 1$, 2, 3, 4, 5, 6 and 24 h data for the second set of analyses. For Cdyn, dPpl, R_L, W_b, HR and RR in Protocol 2, whether there were differences between revatropate and ipratropium in how values changed in the seven time points post-treatment were evaluated using regression. For all but RR an additional quadratic term between parameter values and time was required. As with the other analyses which horse the data were from was entered as a random effect to account for repeated measures.

For all analysis $P < 0.05$ was taken to indicate statistical significance and all analyses were carried out in R (v 2.11.1; 2010 R Foundation for Statistical Computing).

Results

Protocol 1

Prior to all treatments ($T = 0$) horses had significantly increased combined clinical, nostril and abdominal scores, dPpl, R_L and W_b and significantly decreased Cdyn, when compared with data collected during disease remission ($P < 0.035$; Table 1). In addition, prior to ipratropium administration, HR and RR were also significantly increased and decreased, respectively ($P < 0.022$).

Revatropate and ipratropium significantly decreased combined clinical and abdominal scores, RR, HR, dPpl, R_L and W_b ($P < 0.049$, Table 1). These changes were noted after the lowest dose of both drugs, except the reduced heart rate which occurred only after the second dose of revatropate, and the reduced combined clinical and abdominal scores which occurred only after the second dose of ipratropium. While revatropate and ipratropium improved Cdyn, this was significant only with ipratropium ($P < 0.006$). At variable time points following administration of revatropate and ipratropium, RR, HR, Cdyn, dPpl, R_L and W_b were not significantly different from in remission data ($P > 0.053$), while combined clinical and abdominal scores were always significantly higher than those recorded in disease remission ($P < 0.036$;Table 1). Saline inhalation had no significant effects.

None of the treatments altered iris function or significantly altered nostril or gastrointestinal sound scores ($P > 0.170$). However, three horses had reduced gastrointestinal sound scores after administration of revatropate and ipratropium, and some had tacky oral mucous membranes following administration of high doses of revatropate ($n = 2$) and ipratropium ($n = 3$).

Protocol 2

Prior to all treatments ($T = 0$) horses had significantly increased combined clinical, nostril and abdominal scores, dPpl, R_L and W_b and significantly decreased Cdyn, when compared with in remission data ($P < 0.032$; Table 2). HR and RR were also significantly increased prior to ipratropium administration ($P < 0.016$), as was HR prior to saline administration ($P = 0.001$).

Revatropate significantly reduced RR (2 h, $P = 0.012$; Fig. 1a), HR (3 h; $P = 0.033$; Fig. 1b), W_b (1–4 h; $P < 0.003$; Fig. 1c), dPpl (1–5 h; $P < 0.032$; Fig. 1d), R_L (1–5 h; $P < 0.009$; Fig. 1e), abdominal score (from 1 to 3 h inclusive; $P < 0.038$; Fig. 1 g) and combined clinical score (at 1 h; $P = 0.032$; Fig. 1i) (Table 2). Ipratropium significantly reduced RR (at 1, 2, 4 and 5 h; $P < 0.025$), HR (1–24 h; $P < 0.023$), dPpl (1–6 h; $P < 0.008$), R_L (1–6 h; $P < 0.021$) and W_b (1–6 h; $P < 0.005$). Combined clinical, nostril (Fig. 1h) and abdominal scores did not change significantly after ipratropium administration ($P > 0.071$). There was a minor, but significant, increase in Cdyn following administration of revatropate (1–4 h), ipratropium (1–2 h) and saline (1 h) (Fig. 1f; $P < 0.044$). There was also a minor but significant reduction in HR at 5 h after saline administration ($P = 0.049$), but saline had no significant effect on the other variables ($P > 0.056$).

The treatments did not alter mucous membrane moistness or iris function and had no significant effect on nostril or gastrointestinal sound scores ($P > 0.148$). Two horses had reduced gastrointestinal sound score at various time points after administration of revatropate and ipratropium. Finally, there was no significant difference between the efficacy of ipratropium and revatropate in the pattern observed with time for Cdyn, dPpl, RR, HR, W_b and R_L ($P > 0.126$).

Discussion

Inhalation of revatropate and ipratropium markedly and significantly improved airway function in heaves horses, even at the lowest doses used. It is therefore possible that even lower doses of these drugs would also have been effective. Significant improvements in most indices of airway obstruction occurred within 1 h after drug administration, with some of these indices remaining significantly improved at 5 h after inhalation of revatropate and at 6 h after inhalation of ipratropium. The duration of action of