Comparison of two altrenogest preparations in mares: a pilot study

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Abstract

Altrenogest is a synthetic progestin used in reproductive management of horses. Aims were to characterize and compare plasma altrenogest concentrations associated with weekly injectable versus daily oral preparations of altrenogest treatments. We hypothesized that an injectable preparation would not maintain concentrations higher than an oral preparation given once weekly. Two mares with no in vivo source of progesterone received either Treatment A (150 mg intramuscular altrenogest on days 0, 7, and 14) or Treatment B (0.044 mg/kg bodyweight of oral altrenogest daily for 3 weeks). After a 3-week washout period, mares received the opposite treatment for 3 weeks. Blood was collected prior to treatments and on days 0, 1, 4, 7, 8, 11, 14, 15, 18, and 21. High-performance liquid chromatography-tandem mass spectrometry was used for altrenogest assays. Mean minimum plasma concentrations after Treatments A and B were 1.51 ± 0.65 and 2.38 ± 1.53 ng/ml, respectively. Plasma altrenogest concentrations were significantly higher in Treatment A than Treatment B at 24 hours posttreatment (days 1, 8, and 15). No significant difference between plasma altrenogest concentrations was detected on days 4 and 7 of each week. Finding that plasma altrenogest concentrations were higher for Treatment A at all time points except days 7, 14, and 21 supported our hypothesis. An injectable altrenogest preparation may be considered a more practical and safer solution for long-term treatment; based on this study, it was comparable to a proven daily oral altrenogest treatment for a minimum of 4 days.

Keywords: Altrenogest, equine, injectable, oral, plasma

Introduction

Altrenogest is a synthetic progestin commonly used in mare reproduction for behaviour modification, control of estrus, maintenance of pregnancy and uterine quiescence, and induction of lactation in nonparturient mares.[1-4] Such uses require altrenogest treatment ranging from 10 - 120 days. Currently, only 1 oral altrenogest preparation is available in Australia with a demonstrated progestational effect in mares. However, daily oral altrenogest treatment poses risks to personnel who provide treatment, since it is an oil-based preparation that is readily absorbed through skin.[5] Human risks from cutaneous exposure include disrupted menstrual cycles, prolonged pregnancy, decreased libido in men, headaches, fever, abdominal pain, nausea, diarrhea, and rashes.[6] In addition to inherent safety risks to personnel, daily oral altrenogest treatment is logistically difficult and time consuming.[7, 8] Recent research has focused on determining efficacy of other routes of altrenogest treatment. Although intrarectal and intravaginal treatments of altrenogest (0.044 mg/kg) resulted in absorption, plasma concentrations were significantly lower than oral treatment.[5] Doubling oral dose (0.088 mg/kg) was recommended for intrarectal treatment to mares unable or unwilling to receive oral treatment.[9] This treatment resulted in plasma concentrations > 0.5 ng/ml that were maintained for 5.5 hours (range, 3 - 8 hours). Although intrarectal treatment is potentially useful for mares that cannot receive oral treatment, increased handling of this hormone, every 4 - 8 hours, poses a greater health risks to personnel. Consequently, there is a need to ensure a more convenient and safer delivery method.

In recent years, despite previous studies supporting supplementation of $\geq$ 225 mg,[10] Australian Pesticides and Veterinary Medicines Authority approved treatment of injectable preparations of altrenogest (150 mg/500 kg body weight every 5 - 7 days). This reduced frequency of handling may be a safer alternative for personnel. However, there is a need to assess its efficacy or bioequivalence compared to a proven daily oral treatment regimen.

Although pharmacokinetic studies that determined half-life and excretion rates of injectable altrenogest were limited to a single dose treatment, clinical cases traditionally require treatment beyond a single dose. Additionally, there is apparently no documentation of plasma altrenogest

concentrations (PAC) associated with oral altrenogest treatment at 0.044 mg/kg for the recommended treatment period of 15 days.

Objectives of this pilot study were to characterize plasma altrenogest concentrations after oral and injectable preparations over a 3-week period. Secondly, to determine if an injectable preparation is equivalent to an oral preparation for the recommended 7-day treatment period, to ascertain if injectable altrenogest can be used as a safer substitute for oral altrenogest.

Material and methods

This research was conducted at Charles Sturt University (CSU), with institutional Animal Care and Ethics Committee approval (Approval No. 15/037).

Animals

Two healthy mares aged 6 (Horse 1; Cleveland Bay; 570 kg) and 8 (Horse 2; Stockhorse; 458 kg) years, with proven inability to produce progesterone from a luteal source (ovariectomized and hypoplastic ovaries, respectively), were used in this pilot study.

Treatment and blood sampling

This study was conducted between June and August 2015 as a bioequivalence crossover design trial. Both mares received either Treatment A (3 ml of 50 mg/ml intramuscular altrenogest [Ovu-Mate®; Randlab, Chipping Norton, NSW, Australia]) on days 0, 7, and 14) or Treatment B (0.044 mg/kg bodyweight daily oral altrenogest [Regumate®; Intervet, Bendigo, VIC, Australia]) for 3 weeks. After a 3-week wash-out period, mares received the opposite treatment for a further 3 weeks. Venous blood was collected into heparinised tubes (10 ml) for plasma altrenogest assay, prior to treatments (day 0) and on days 1, 4, 7, 8, 11, 14, 15, 18, and 21.

Assay and data analysis

Samples were analyzed for PAC using high-performance liquid chromatography-tandem mass spectrometry (LCMS-MS) in a commercial laboratory (Pia Pharma Pty Ltd), according to methods previously reported.[11] Quantitative analysis was based on an internal standardization using $C_{21}H_{24}O_2$, gestrinone with a molecular weight of 308.421 g/mol. In comparison, altrenogest structure is $C_{21}H_{26}O_2$ with a molecular weight of 310.437 g/mol. A calibration curve, prepared with concentrations ranging from 1 to 50 ng/ml altrenogest, was used for quantitation. Correlation coefficient (r) was 0.9996 which met in-house criteria ($r > 0.99$). The analyst was blinded to treatments. Differences in PAC between treatment routes were analyzed for days 1, 4, and 7 of each week (days 1, 4, 7, 8, 11, 14, 15, 18 and 21) using a series of paired Student's t-tests. Interactions between PAC and time were tested by repeated measures analysis using MIXED procedure of SAS.

Results

Daily oral altrenogest treatment resulted in minimum PAC between 0.87 and 4.17 ng/ml (Table). Weekly altrenogest injections resulted in minimum PAC ranging from 0.64 to 2.79 ng/ml over the treatment period (Table).

Table. Plasma altrenogest concentrations (ng/ml) over 21-day treatment period

	Treatment group	Day 0	Day 1	Day 4	Day 7	Day 8	Day 11	Day 14	Day 15	Day 18	Day 21
Horse 1	**A**	< 0.5	13.16	2.38	0.64	14.37	4.31	1.6	17.2	4.05	1.31
	B	< 0.5	1.79	3.36	3.8	3.57	3.36	3.89	3.25	4.17	3.63
Horse 2	**A**	< 0.5	13.29	3.86	1.2	10.91	3.21	1.51	7.52	3.09	2.79
	B	< 0.5	0.96	1.06	0.87	1.03	0.93	1.13	1.02	0.87	0.96

A. Injectable altrenogest once weekly (days 0, 7, and 14) intramuscular; B. Oral altrenogest once daily for 21 days

Mean minimum PAC following Treatment A and Treatment B were 1.51 ± 0.65 and 2.38 ± 1.53 ng/ml, respectively (mean ± SD). Mean PAC for Treatment A in Horses 1 and 2 over the trial period were 6.56 ± 6.46 and 5.26 ± 4.32 ng/ml and for Treatment B in Horse 1 and 2 were 3.42 ± 0.68 and 0.98 ± 0.09 ng/ml.

Plasma altrenogest concentrations in Treatment A was significantly higher than Treatment B at 24 hours posttreatment (days 1, 8, and 15 of the trial period). Posttreatment, with weekly altrenogest, PAC markedly decreased between days 1 and 4, and days 4 and 7 over each treatment week (Figure).

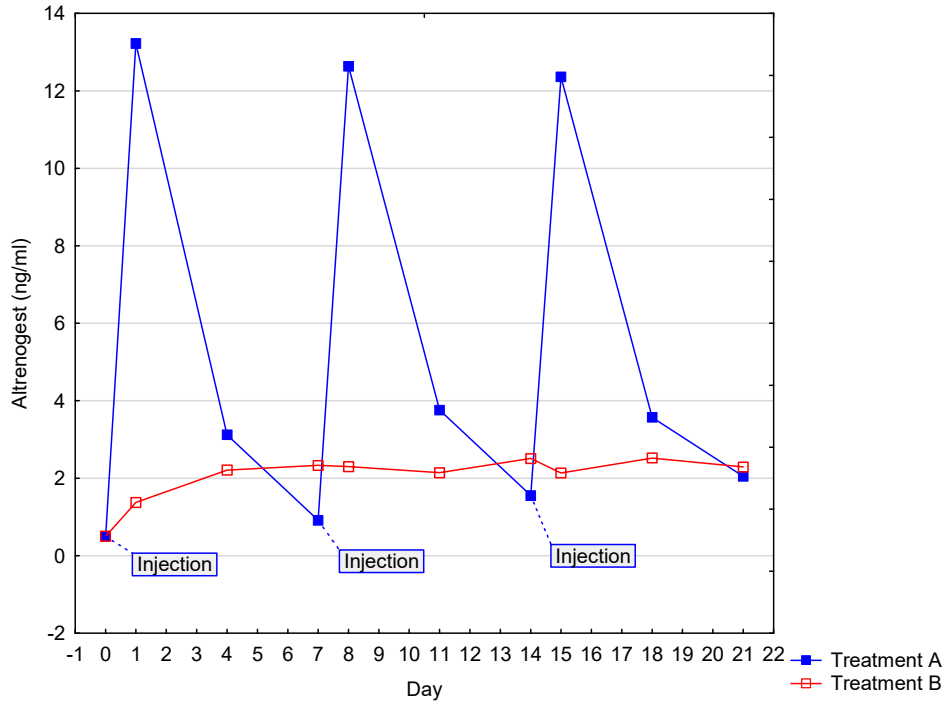

Figure. Mean plasma altrenogest concentrations over the 3-week period. Treatment A: intramuscular altrenogest treatment (Injection) once weekly on days 0, 7, and 14. Treatment B: oral altrenogest treatment once daily for 21 days.

Mean PAC from Treatment A was greater than that of Treatment B on day 4 (3.48 ± 0.72 and 2.29 ± 1.5 ng/ml, respectively). By day 7 of each week, mean PAC of Treatment A were below Treatment B (1.51 ± 0.65 and 2.38 ± 1.53 ng/ml). No significant difference between PAC of selected preparations was detected on days 4 or 7 of each week.

Discussion

Injectable alternogest resulted in an immediate increase in PAC > 10 ng/ml at 24 hours after treatment. By 4 days posttreatment, PAC markedly decreased and were > 2 ng/ml. Mean PAC were higher for a minimum of 4 days in injectable altrenogest compared to oral altrenogest. On day 7 of each week, PAC of injectable altrenogest were lower than oral altrenogest (1.51 ± 0.65 and 2.38 ± 1.53 ng/ml, respectively). Similar results were observed when weekly injectable altrenogest (Readyserve® 150 mg/week) was used.[12] Mean PAC were highest ~ 24 hours after treatment and were > 1 ng/ml on day 4 and < 0.5 ng/ml at 6.2 days (148h). An in vivo assessment of Readyserve® at the same dosage used in this study was not reliable in supressing estrus in all mares for the full 7-day treatment window. Comparable to this, our findings indicated that concentrations associated with injectable altrenogest preparations were higher than oral altrenogest for 4 days, but may not effective between days 5 and 7 posttreatment in mares. Other injectable altrenogest, not readily available in Australia, have been successful in suppressing estrus and ovulation in mares treated for up to 33

days.[10] However, the recommended dosage is 1.5 - 3.3 times higher than the dosage used in our study. Therefore, further investigation with increased dosing regimens would be beneficial, as it may result in higher PAC, more effective suppression of estrus and reduced frequency of treatments, thereby minimising human contact and associated risks.

For both treatments, Horse 1 had higher PAC than Horse 2. Interestingly, Horse 1 was considered a body condition (fatness) score of 2 (thin), whereas Horse 2 was considered a body condition score of 4 (fat) on a scale of 0 to 5. Altrenogest was detected in liver, fat, kidneys and muscle after treatment. Liver had the highest tissue concentrations of altrenogest posttreatment, followed by fat.[13] To date, there are no studies in horses that indicated whether high body condition influenced circulating plasma concentrations of progestogens. However, an influence was noted in cattle, rats and humans.[14-16] A horse's body condition could influence circulating plasma concentrations, particularly if altrenogest is deposited in fat. As altrenogest is marketed with a weight-dependent dosage, further research is warranted to ensure that body condition does not adversely influence altrenogest efficacy.

During the 3-week trial period, mean minimum concentrations of altrenogest for both preparations had an increasing trend. At the end of the first full treatment period for each preparation, (7 days for injectable and 24 hours for oral), mean minimum concentrations for Treatments A and B were 0.92 and 1.38 ng/ml, respectively. However, at the end of the 3-week trial period, mean minimum concentrations for Treatment A and Treatment B were 2.05 and 2.30 ng/ml, respectively. Although our sample size was not large enough to identify a significant difference, this observation may be an indication of a cumulative effect worthy of further research. A cumulative effect following long-term altrenogest has not been reported in horses. However, during prolonged altrenogest treatment in pigs, an accumulation in blood plasma has been demonstrated.[13] Further research into the possibility of a cumulative effect of long-term altrenogest treatment could be useful to determine if minimum concentrations of a weekly injectable preparation would eventually maintain concentrations comparable to a daily oral preparation.

There were several limitations associated with this pilot study. A decision was made to use mares with no in vivo source of progesterone, which are scarcely available, and resulted in a sample size of only 2 mares. A larger sample size would have provided a better indication of mean and minimum PAC after treatment, allowing for significant differences to be discriminated. Restrictions on sampling frequency did not allow documentation of peak concentrations. However, minimum concentrations associated with both treatments were characterized, as sampling occurred immediately prior to treatments.

Results from this preliminary study characterized PAC during treatment and provided evidence that injectable altrenogest was comparable to daily oral altrenogest for a minimum of 4 days, but may not be comparable or effective between days 5 and 7 posttreatment. This should be taken into consideration if using injectable altrenogest as a substitute for oral altrenogest in mare reproductive management, as minimum target PAC are yet to be determined for either pregnancy management or estrus suppression. Further research with a larger number of animals is required to determine bioequivalence of these 2 preparations.

Conflict of interest

None to declare.

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