Plasma altrenogest concentrations in mares after intrarectal or intravaginal administration

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Abstract

Altrenogest (Regu-Mate®) is labelled for daily oral administration and is used in mares to suppress estrus behavior, maintain pregnancy and synchronize estrous cycles. It is a very potent progestin, with potential risks associated with transdermal absorption for persons handling or administering this compound. The objective was to determine plasma concentrations of altrenogest following rectal or vaginal administration in mares. Altrenogest (0.044 mg/kg) was administered once daily for 5 days; orally, vaginally, and transrectally in 7, 5 and 5 mares, respectively. Transrectal palpation and ultrasound examinations were performed on Days 1, 3, and 5 to assess cervical tone, endometrial edema, and ovarian structures. Venous blood was drawn at time 0 (prior to drug administration) and at 30 and 60 minutes after drug administration. To determine altrenogest concentrations, plasma from each sample was analyzed via ultra-performance liquid chromatography coupled to a triple quadruple mass spectrometer. Although absorption of altrenogest occurred after vaginal or oral administration, plasma altrenogest concentrations were less ($p < 0.05$) than those of orally treated mares. Additional studies are needed to determine if plasma altrenogest concentrations after rectal or vaginal administration are sufficient to suppress estrus behavior or maintain pregnancy.

Keywords: Estrus suppression, rectal, vaginal, progestin, progestogen, altrenogest

Introduction

Progestins are used in equine reproductive medicine to prevent expression of unwanted estrus behavior, aid in estrus synchronization, hasten onset of cyclicity in late transition mares, aid in maintenance of pregnancy, or treat placentitis.[1-9] Oral altrenogest (Regu-Mate®, Intervet/Merck Animal Health, Madison, NJ) treatment effectively binds to the progesterone receptor in the mare at the label dose (0.044 mg/kg), is safe and efficacious for extended intervals in mares and does not affect fertility.[4,6,10] Oral administration of altrenogest resulted in prompt suppression of estrus behavior that continued until cessation of administration, with estrus occurring within 10 days.[11] Orally administered altrenogest (0.044 mg/kg) reached maximum plasma concentrations within 15 to 30 minutes after administration in 70% of horses, within 90 minutes in another 20% of horses and within 180 minutes in the remaining 10%.[12] Mean peak plasma altrenogest concentration was 35 ng/ml (range, 23 - 75).[12]

Although oral is the primary route of administering altrenogest, this poses some risk to persons administering it, as it is readily absorbed, through intact skin. Human risks associated with cutaneous exposure to altrenogest include an altered menstrual cycle or prolonged pregnancy. People with conditions such as thromboembolic disorders, cerebral-vascular or coronary artery disease, known or suspected breast carcinoma, estrogen-dependent neoplasia, undiagnosed vaginal bleeding, oral contraceptive-induced tumors, or liver disease are advised not to handle this product.

In addition to potential health implications for persons administering the drug, oral administration can be difficult for owners. The requirement for daily oral administration, often for extended intervals, becomes difficult if a horse develops avoidance behaviors such as tossing their head to resist treatment or spitting out the drug. Regarding the latter, the administrator attempts to prevent drug loss by holding the horse's mouth shut and raising its head, making them vulnerable to drug exposure, even if they are wearing rubber gloves. Another option for administration is adding the oil-based drug to the horse's grain; however, loss of drug in the feed bucket, resulting in inadequate dosing, is a practical concern. Lastly, there are instances when oral administration to a hospitalized pregnant mare is contraindicated, i.e. refluxing anterior enteritis, intestinal obstruction or ileus, esophageal disease, or facial bone fractures.[13] In clinical cases such as these, injectable progestogen formulations are ideal, although access may be delayed if the product is not readily available.

Alternative drug administration routes have yielded favorable results in various species; an added benefit was increasing duration of drug exposure to mucosal surfaces.[14-17] For example, metronidazole was rapidly absorbed after rectal administration in horses. Although not superior to oral or intravenous administration, it is a viable, alternative route, as the bitter taste reduces feed intake, even if flavor is added.[14] Acetylsalicylic acid, used for treatment and prevention of arterial thrombosis in horses, has better bioavailability after rectal versus intragastric administration.[15] Diazepam, frequently used for seizures, was rapidly absorbed and active after rectal administration in dogs.[16] Levetiracetum, also used for seizures in dogs, exceeded minimum target concentrations within 10 minutes after rectal administration and sustained them.[17]

Regarding reproductive drugs used in both veterinary and human medicine, not only is intravaginal administration of progesterone or prostaglandins (e.g. PGE_1, misoprostol and PGE_2, dinoprostone) a major method of drug delivery, the efficacy of the drug is also enhanced.[18-21] Intravaginal drug delivery is routinely used for estrous cycle management in ruminants, e.g. controlled internal drug-release (CIDR) devices, progesterone-releasing intravaginal devices (PRIDs), and vaginal sponges.

Drug administration via rectal or vaginal routes prolongs drug exposure to mucosal surfaces. Intravaginal or intrarectal administration routes is expected to be advantageous for administration of altrenogest in horses. These should be viable options for horse owners who are unable to use injectable progestogens due to needle-shyness, for horses that develop injection site reactions, and/or horses averse to oral treatments. The objective was to determine plasma altrenogest concentrations after rectal or vaginal administration.

Materials and methods

Animals and structure

Seventeen healthy mares (14 Quarter Horse, 3 Thoroughbred), from 4 - 24 years were used. Mares were housed at a university research farm, kept on pasture and fed concentrate twice daily with *ad libitum* access to hay and water. All mares were weighed on Day 1 (D1) to ensure accurate drug dosing. Horses were handled in accordance with the US Department of Agriculture Guide for Care and Use of Agricultural Animals in Research (ACUP VM-15-28).

Experimental groups

Assignment of mares to 3 groups was at random. Treatments were administered at the same time each day. Five mares received intrarectally (0.044 mg/kg[12] altrenogest, every 24 hours for 5 consecutive days [rectal group, RG]). Five mares received intravaginally (0.044 mg/kg altrenogest, every 24 hours for 5 consecutive days [vaginal group, VG]). A third group, which initially consisted of 2 mares that served as oral-administered controls, received altrenogest orally (0.044 mg/kg) every 24 hours for 5 consecutive days (oral group, OG); this group later expanded to a total of 7 mares after addition of 5 mares to serve as OG controls. Expansion of the oral group occurred 5 months after the initial work.

Routes of administration

For the OG group, altrenogest was deposited into the mare's oral buccal pouch with an oral dose syringe. Mares were closely observed for 1 to 2 minutes thereafter and drug loss/oral expulsion noted. Mare's heads were tied to a fence rail to prevent grazing. Any drug loss during the next 60 minutes was recorded.

For the RG group, manure was not evacuated from the rectum before treatment, to simulate treatment by non-veterinary personnel. A 15 cm pipette was inserted ~5 cm anterior to the anal sphincter and a pre-loaded syringe attached to inject the drug; back flow or resistance during pipette insertion/drug injection was recorded. If a mare defecated within 1 hour after rectal administration, the time was recorded.

Prior to daily vaginal administration of altrenogest, the vulvar mucosa was examined for signs of irritation and the vulvar commissure was cleaned once using Ivory liquid Soap and water. Vaginal drug administration was accomplished by wearing a sterile sleeve and manually guiding a 15 cm pipette through the vulva and vestibule and into the caudal vagina. Any back flow or resistance was recorded.

Blood sampling

Each day, jugular venipuncture was performed 3 times[12] on each mare after administration of altrenogest; once prior to administration, 30 minutes after administration, and 60 minutes after administration. Approximately 3 to 5 ml of blood was collected via a vacutainer into blood tubes containing EDTA. All blood samples were centrifuged at 2000 x g for 10 minutes and plasma was decanted and frozen at -80°C until assayed.

Reproductive examination

Transrectal ultrasonographic (Sonosite M-Turbo®, Fujifilm, 7.5 MHz, linear-array transducer) examinations were performed on Days 1, 3, and 5. Cervical tone was graded on an A to C scale with A indicating a relaxed, open cervix and C indicating a tight, closed cervix. Edema was graded from 0 to 3 with a score of 0 indicating no edema and a score of 3 indicating heavy edema. The presence of fluid was noted and when present, echodensity, location and largest pocket size were noted. Ovarian follicle size and the presence of a corpus luteum (CL) were recorded. Mares were classified as being in estrus (presence of endometrial edema, A/open cervix) or diestrus (lack of endometrial edema, C/closed cervix, presence of a CL).

At the conclusion of the 5-day treatment regimen, a speculum examination was performed rectally or vaginally, as dictated by treatment group, to detect evidence of mucosal irritation as a result of administering an oily compound (Neobee® M-5, Regu-mate® vehicle).

Hormone assay

Plasma altrenogest concentrations were determined by ultra-performance liquid chromatography coupled to a triple quadruple mass spectrometer (UPLC-MS). The UPLC-MS system consisted of an Agilent 1290 system coupled with an Agilent 6460 Triple Quad Mass Spectrometer (Agilent Technologies, Santa Clara, CA) equipped with an electrospray ionization (ESI) interface. Nitrogen gas was used as the drying (10 l/min at 350°C), nebulizing (45 psi) and collision gas. The mass spectrometer was operated in the positive mode and mass transitions were monitored using multiple reaction monitoring (MRM).

The standard curve, ranging from 3.9 to 1000 ng/ml, was generated by fortifying mare plasma with known amounts of altrenogest reference standard and Trenbolone internal standard (Sigma-Aldrich, St. Louis, MO) that were prepared in 50:50 (v/v) acetonitrile/distillated water and acetonitrile respectively. Standard curves were accepted if coefficient of determination (r^2) was at least 0.999 and predicted concentrations were within ± 20% of actual concentrations.[22] The UPLC-MS assay was validated for mare plasma, with the following criteria: linear correlation coefficient (r^2) for altrenogest was 0.999; limit of detection was 3.91 ng/ml; lower limit of quantification was 3.91 ng/ml; and upper limit of quantification was 1000 ng/ml.

Statistical analyses

Analysis of variance was conducted to compare the plasma altrenogest concentrations. A repeated measures model was used and means compared with protected pairwise comparisons.

Results

Altrenogest was absorbed after all 3 routes of administration. Mean plasma altrenogest concentrations at 0, 30, and 60 minutes over the 5-day treatment period are shown (Figure 1). Over the entire treatment period, plasma altrenogest concentrations, were similar in RG and VG mares but lower ($p < 0.05$) that in OG mares (Figure 2).

Control mares

In OG mares, mean maximal plasma altrenogest concentration was 117.32 ng/ml (range, 31.0 - 366.5). Overall, 35 doses of altrenogest were administered to OG mares; on 12 occasions, plasma concentrations peaked at 30 minutes after administration and on 23 occasions, they peaked at 60 minutes.

Rectal administration mares

For RG mares, mean maximal plasma concentration was 35 ng/ml (range, 5.8 - 100.5). During the treatment period, 25 doses of altrenogest were administered to OG mares. Following the administration, on 11 occasions plasma concentrations peaked at 30 minutes and on 14 occasions peaked at 60 minutes.

Vaginal administration mares

For VG mares, mean maximal plasma concentration was 18.0 ng/ml (range, 5.4 - 61.3). All mares always had maximum concentrations at 60 minutes after administration. A recurring challenge in 3 VG mares was moderate difficulty to guide the 15 cm pipette through the vestibule-vaginal sphincter. Consequently, drug was deposited within the vestibule to mimic actions of non-veterinary personnel. Subsequently, altrenogest was expelled from the vulva shortly thereafter, a problem made worse by clitoral eversion that occurred soon after vaginal drug administration. Thus, in these 3 mares, vestibular deposition and clitoral eversion resulted in decreased contact time and drug loss.

Adverse effects

Each administration was accompanied by assessment for adverse effects, including monitoring for increased respiration rate, unease, vaginal and/or rectal discomfort, and vulvar/anal redness and discharge were monitored for. No adverse effects were noted following administration of altrenogest by any route.

Transrectal ultrasonography

All mares were cyclic during the 5-day treatment period. Of the vaginally treated mares, 4 of the 5 mares were in diestrus and 1 was in estrus. Of the rectally treated mares, 2 were in estrus and 3 in diestrus. Of the orally treated mares, 2 were in diestrus, 4 were in estrus, and 1 ovulated between Days 1 and 3 of the study.

Discussion

Oral absorption of altrenogest was variable among mares. Mean maximal plasma altrenogest concentration (117.3 ng/ml), after oral administration, was higher than reported (35 and 16 ng/ml)[12,13] despite a lower dose (0.044 versus 0.088 mg/kg[13]). The range for peak concentrations in OG mares, 30.2 - 366.5 ng/ml, was also higher than reported (23 - 75 ng/ml).[12] All peak plasma sample concentrations in OG mares were at or above maximal plasma concentrations reported.[12] No drug loss via oral expulsion was noted in any OG mares. Perhaps preventing access to feed and water for 60 minutes after treatment increased concentrations.

Altrenogest was absorbed via the rectal mucosa into systemic circulation. Plasma altrenogest concentrations were higher in the RG versus the VG, but both were less than those in the OG. There was individual mare variation (5.8 - 100.51 ng/ml) in peak plasma altrenogest concentrations following rectal administration. Mean maximum plasma concentrations in rectally administered mares was 35 ng/ml, similar to a reported for geldings,[12] but much higher than reported for mares (2.54 ng/ml).[13]

Altrenogest was absorbed via the vaginal mucosa into systemic circulation. However, systemic plasma concentrations of vaginally administered altrenogest were vastly lower when compared to orally administered mares, due to suboptimal drug placement, rapid loss by expulsion, and perhaps inadequate absorption. Mean maximum plasma altrenogest concentrations after vaginal administration was 18 ng/ml (range, 5.4 - 61.3), lower than maximum concentrations (23 -75 ng/ml) reported following oral administration.[1]

Rectal and vaginal administrations resulted in detectable systemic concentrations of plasma altrenogest; however, concentrations were not as high as oral administration of altrenogest. Incomplete and variable absorption of altrenogest may have been due to a number of factors, including drug placement, external elimination, drug dosing, absorption barriers, and inadequate time for absorption.

The location of drug placement can affect drug absorption. Administration of altrenogest with a 15 cm pipette allowed for the entire dose to be deposited in the caudal rectum, allowing absorption to occur via the pudendal vein into the caudal vena cava, thus bypassing the liver and first-pass metabolism. Defecation is expected to reduce drug absorption; an obvious drawback of rectal administration of drugs in a horse is inability to delay defecation. Despite this, rectal administration of the drug without manure evacuation was performed to simulate an owner administering the drug at home, as we would not recommend owners doing transrectal palpation, due to risk of injury to the horse and owner. Three of the 5 mares defecated within 30 minutes after drug administration on Day 2 of the study which likely resulted in a reduction of plasma altrenogest concentrations for that day, since only one of those 3 mares achieved a concentration above the previously reported 23 - 75 ng/ml concentration after oral administration.[12]

The vestibule of the mare is ~10 cm in length, whereas the vagina is ~8 cm in length. Using a 15 cm pipette, altrenogest was deposited within the posterior vagina. Vaginal administration via pipette was more difficult in some mares than rectal administration and should not be recommended for owners to attempt. The presence of a tight vestibule-vaginal sphincter in some mares made it more difficult to reach the vagina; altrenogest was subsequently deposited in the vestibule and was expelled from the vulva via clitoral eversion. Whereas drug loss appeared to occur in 3 of the 5 mares that were visually recorded to lose the drug via the vulva, the remaining 2 mares still failed to achieve statistically significant altrenogest plasma concentrations when compared to orally dosed mares.

The established oral dose of altrenogest is 0.044 mg/kg,[23] but minimum target plasma concentrations of altrenogest are not determined for suppression of estrus behavior or maintenance of pregnancy. A dose of 0.022 mg/kg, was adequate for maintaining pregnancy, at least up until day 40 of gestation.[3] Even though we demonstrated altrenogest was absorbed in both alternative routes, it is not known whether concentrations of altrenogest absorbed transrectally or transvaginally are adequate for the desired clinical effects.

Absorption barriers are present in both the rectum and the vagina. Despite the presence of feces, plasma concentrations in the RG group were higher than reported wherein a higher dose of altrenogest (0.088 mg/kg) was used.[13] Absorption may have also been impacted by the endocrine status in the VG mares, since thickness of vaginal mucus or vaginal epithelium (fluctuating by the stage of the estrous cycle) could be a barrier to absorption. The vagina is lined with stratified squamous epithelium, with its degree of secretory activity and thickness fluctuating with endocrine status; progesterone and estrogen can influence the structure and function of vaginal epithelium, since receptors for these hormones are present in the vagina.[24] In the estrogen-dominated phase, vaginal cornification ensues and results in an increased number of existing cell layers; vaginal mucus is more voluminous but less viscous.[25] During diestrus, mucus viscosity increases, however, volume decreases.[25] Among the 5 mares treated vaginally, 4 were in diestrus and 1 was in estrus, making it less likely that decreased absorption was due to a thickened vaginal epithelium. Interestingly, it was difficult to pass the pipette in the mare in estrus and drug loss was noticed after clitoral eversion, but perhaps this was simply due to individual anatomical variation.

Conclusions

In mares, rectal or vaginal administration of altrenogest are not recommended as alternate methods to oral administration, as plasma altrenogest concentrations 60 minutes after administration were significantly lower compared to mares that had oral administration. Further studies are needed to determine if plasma altrenogest concentrations achieved after rectal or vaginal administration suppress estrus behavior or maintain pregnancy.

Conflict of interest

The authors have no conflicts of interest.

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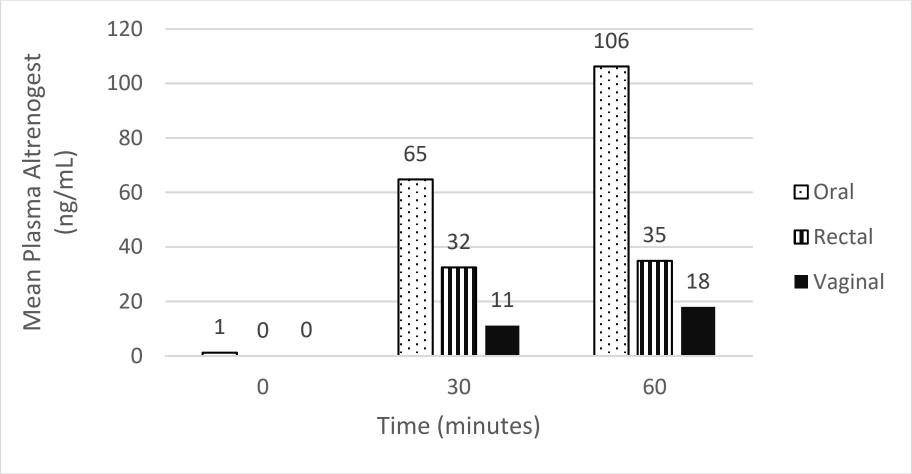

Figure 1. Mean plasma altrenogest concentrations (over the 5-day treatment period) at 0, 30, and 60 minutes after administration (oral, rectal or vaginal).

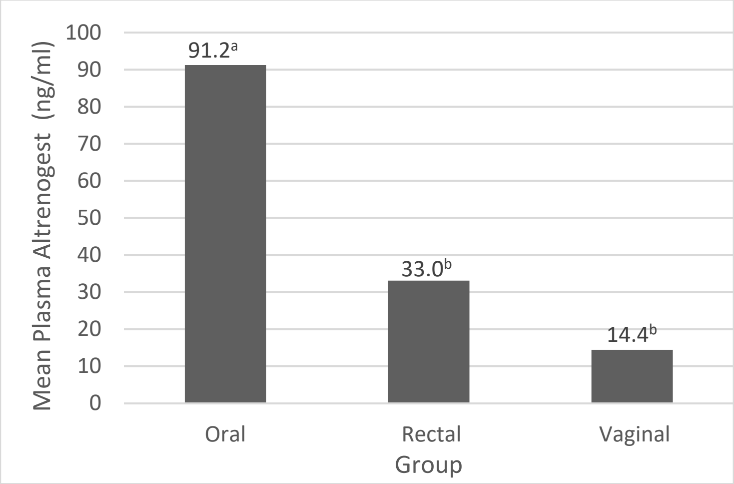

Figure 2. Mean plasma altrenogest concentrations, reported by group, for the entire treatment period.
^{a,b}Means without a common superscript differed ($p < 0.0001$).