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Mid-gestation pregnancy is not disrupted by a 5-day gastrointestinal mucosal cytoprotectant oral regimen of misoprostol

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Summary

Reasons for performing study: To investigate effects of a 5-day oral misoprostol regimen recommended for use in horses as a gastrointestinal mucosal cytoprotectant during colic on mid-gestation pregnancies.
Objectives: To monitor cervical tone, ultrasonographic characteristics of the uterus, cervix and conceptus, as well as serum progesterone and oestrone sulphate concentrations, and observations of general health, behaviour and comfort of mid-gestation mares given a 5-day course of misoprostol or control treatment.
Methods: Eleven light horse and pony mares with known breeding dates were administered 5 μg/kg bwt misoprostol orally, twice daily for 5 days. General health and pregnancy status were monitored daily during treatment via general physical examination, as well as palpation and ultrasonography *per rectum* of the uterus, cervix and conceptus. Jugular serum was obtained during and for 5 days following treatment for assay of progesterone and oestrone sulphate concentrations. Additionally, daily 12 h video samples of the mares were obtained to evaluate behaviour and comfort.
Results: All findings, including cervical tone, ultrasonographic characteristics of the uterus, cervix and conceptus, as well as progesterone and oestrone sulphate concentrations, and observations of general health, behaviour and comfort, were similar during misoprostol and control treatment.
Conclusions: Treatment of pregnant mares with a gastrointestinal mucosal cytoprotectant regimen of oral misoprostol for 5 days did not disrupt pregnancy, nor adversely affect the general health and comfort of these mares. Additional investigation of treatment at earlier and later stages of gestation, for longer-term treatment, as well as evaluating neonates for developmental disturbances, would add further information on safety of misoprostol during gestation.
Potential relevance: These results provide some assurance of safety of a 5-day gastrointestinal mucosal cytopretectant regimen of oral misprostol in mid-gestation pregnant mares.

Keywords: horse; misoprostol; mare; pregnancy; abortion

Introduction

Prostaglandins have been found effective in inhibiting secretion of gastric acid [1], promoting secretion of mucus and bicarbonate [2,3] and providing cytoprotection of gastrointestinal mucosa in various mammalian species [4–6]. In horses, misoprostol, a synthetic methyl ester analogue of prostaglandin E_1 (PGE_1), has been used for treatment of gastro-oesophageal or gastroduodenal ulcers [7–9] and evaluated for benefit to compromised intestinal mucosa, as in the case of ischaemic colic [10]. In women, misoprostol has been used effectively for inducing first-trimester abortions in combination with mifepristone [11] and causes an increase in uterine tone and contractions [12,13] via a variety of routes (oral, buccal, sublingual, vaginal and rectal administration). In goats, oral misoprostol given at doses intended to induce parturition at term effectively induced parturition within a mean of 131.8 h from the first treatment [14].

The safety of oral misoprostol regimens in pregnant mares is therefore questionable. The purpose of this study was to evaluate the effects of a 5-day oral regimen of misoprostol, which is currently used as a gastrointestinal mucosal cytoprotectant in horses with colic, on mid-gestation pregnancies of mares.

Materials and methods

Experimental animals

Eleven pregnant mares with known breeding dates were used. These included 4 light horse mares (Thoroughbred, Thoroughbred crossbred and Morgan crossbred, 10–15 years of age) and 7 Shetland-sized pony mares (2–3 years of age). These animals were resident teaching and research animals at the Hofmann Center of the University of Pennsylvania School of Veterinary Medicine at New Bolton Center. Horse mares were housed in individual stalls with free-choice hay and water during the day and together at pasture at night. Pony mares were housed in stalls and maintained on free-choice grass hay and water. The study was performed with approval and under guidelines of the Institutional Animal Care and Use Committee of the University of Pennsylvania.

Experimental design and treatment protocols

The study was conducted in 4 replicates over a period of 9 weeks from 22 October to 17 December 2009, as the mares under study reached mid-gestation. Four mares received a round of control treatment and measures that preceded their treatment replicate such that *Replicate 1* included one misoprostol-treated and one control-treated mare that subsequently served as a misoprostol-treated mare in *Replicate 2*; *Replicate 2* included 5 misoprostol-treated and 2 control-treated mares, one of which served as a misoprostol-treated mare in *Replicate 3*; *Replicate 3* included 3 misoprostol-treated mares and one control-treated mare that served as a misoprostol-treated mare in *Replicate 4*; and *Replicate 4* included 2 misoprostol-treated mares. Mares were between 94 and 112 days gestation (mean 105 days) at the start of control treatment and between 100 and 129 days gestation (mean 117 days) at the start of misoprostol treatment.

Misoprostol (5 μg/kg bwt; misoprostol 200 μg per tablet)[a] was administered orally by dose syringe twice daily for 5 days. Tablets were crushed and mixed with corn syrup and water. Control treatment consisted of a similar volume of corn syrup and water.

Evaluation of health, comfort and pregnancy status

Beginning the day before the start of misoprostol or control treatment and continuing through Day 5 of treatment, mares were examined by palpation and ultrasonography *per rectum*. Cervical tone, fetal fluid character, integrity of the uteroplacental unit at the caudal uterine body, and fetal activity were recorded daily. On each of those days, a serum sample was obtained for progesterone and oestrone sulphate radioimmunoassay (New York State Animal Health Diagnostic Center, Cornell University). Each mare was examined daily, recording temperature, heart rate, respiratory rate and vulvar appearance (discharge). Manure consistency, appetite and

water consumption when in stalls were observed and any changes noted. Mares were also directly observed frequently (up to several times per hour) by animal care staff in the barn throughout the day and evening hours, noting any signs of discomfort.

During treatment, mares were videorecorded in stalls from 06.00 to 18.00 h following each treatment status. Videotapes were viewed by an experienced technician who remained blind to treatment to scan for any signs of discomfort (pawing, rolling, sweating, abdominal straining and looking at flanks) and to record daily time budgets (percentage of time spent eating, drinking, standing at rest, recumbent at rest and standing alert) and daily activity pattern.

All pregnancies were terminated 3–8 weeks after completion of misoprostol treatment. Mares were examined again by palpation *per rectum* and ultrasonography *per rectum* as described above prior to pregnancy termination.

Results

All pregnancies were maintained with no apparent change in cervical tone, fetal fluid character, integrity of the uteroplacental unit at the caudal uterine body, or fetal activity through the 5 days of treatment and when examined again at 19–54 days after misoprostol treatment, prior to pregnancy termination. Fetuses were examined at the time of elective termination of pregnancy and appeared grossly normal for stage of gestation.

During both misoprostol and control treatments, all mares maintained serum progesterone concentrations within published ranges for the stage of gestation for normal pregnancies [15,16]. Mean serum progesterone concentrations obtained 24 h following the final days of treatment (Days 4 and 5) were not significantly different from the mean of the 2 daily baseline samples obtained immediately prior to treatment (Student's paired *t* test, $P>0.10$).

Serum oestrone sulphate concentrations were reported in a semi-quantitative fashion, as >100 ng/ml (an indication of fetal viability) or as a quantified value in nanograms per millilitre when <100 ng/ml. For the 7 pony mares, oestrone sulphate levels of all samples were >100 ng/ml. For one horse mare, serum oestrone sulphate concentrations were below 100 ng/ml for the baseline days, as well as on Day 1 and Day 5 during the control treatment round, but serum oestrone sulphate concentrations remained above 100 ng/ml for the duration of misoprostol treatment. For the remaining 3 horse mares, oestrone sulphate concentration was below 100 ng/ml for the baseline and for the initial treatment days, but subsequently increased to >100 ng/ml by Day 3 or 4 of misoprostol treatment.

Rectal temperature, heart rate, respiratory rate, vulvar appearance, appetite and water intake remained within normal limits. With the exception of 2 pony mares, manure consistency remained normal during the treatment time periods. The 2 pony mares, one control and one misoprostol treatment, simultaneously experienced softer manure during transition from grass pasture to a hay diet at the start of the study.

On video records or by direct observation, no signs of mare discomfort were observed. All time budgets and general behaviour patterns were within normal limits, with no differences between misoprostol-treated and control mares, and between baseline and treatment days within mares [17].

Discussion

Using a treatment regimen previously shown to reduce gastric pH and free acid contents in the horse [7], no evidence of adverse effects of misoprostol treatment on mid-gestation pregnancies of mares was observed. Cervical tone, ultrasonographic character of the conceptus, progesterone and oestrone sulphate concentrations, as well as observations of general health and comfort, all revealed no differences between misoprostol-treated and control mares.

These pregnancies were purposefully terminated at 3–8 weeks following the completion of misoprostol treatment. Serum progesterone and oestrone sulphate concentrations sampled at that time, as well as reproductive examination, indicated no disruption of fetal well-being. Evaluation of the aborted fetal tissues indicated no gross physical abnormalities. Further work monitoring pregnancies to term following misoprostol treatment, as well as evaluating offspring through development, would provide valuable additional information on the safety of this short-term misoprostol treatment. This work was limited to mid-gestation, which in our clinic is a common stage of pregnancy presenting with colic for which this treatment is considered. Investigation of treatment during earlier or later stage gestation would also add valuable information.

In man, there are a number of known adverse effects of oral misoprostol administration. Nausea and vomiting, as well as an increase in intestinal transit rate with resultant diarrhoea and abdominal pain, are commonly reported adverse effects. Two milligrams of PGE_1 taken orally in 4 healthy volunteers resulted in diarrhoea in all 4 subjects [18]. In canine patients, the administration of oral misoprostol also resulted in 4 of 10 treated patients developing diarrhoea within 24–36 h from the initiation of treatment [19]. None of the 11 mares in this study showed any observable signs of general discomfort or gastrointestinal-related adverse effects during or following misoprostol treatment. A likely reason for the difference in observed adverse effects was that the canine patients were administered 15 μg/kg bwt orally, compared with 5 μg/kg bwt in our mares. In 1985, Hunt and Gerring [20] reported decreased electrical and mechanical activity of the gastrointestinal tract of ponies after intravenously administered PGE_1 (25–75 ng/kg bwt/min for 120 min). While diarrhoea was not a reported adverse effect of misoprostol administration in that study, PGE_1 administration did lead to a dose-dependent decrease of gut sounds, with increasing doses of misoprostol causing greater patient discomfort from what was attributed to be a probable increase in bowel distention from the accumulation of gas or fluid.

In women, oral misoprostol ingestion for the purpose of pregnancy termination is suspected as a cause of Moebius syndrome in infants [21,22]. Moebius syndrome includes facial nerve paralysis resulting from developmental hypoplasia of the cranial nerve nuclei, and in some cases also skeletal, cardiovascular, craniofacial and/or limb defects. This syndrome is suspected to be the result of a transient ischaemic/hypoxic insult to the developing fetus, due to uterine contractions and vasoconstriction of uterine and umbilical vessels [23,24]. Studies indicate that in Brazil, where elective termination of pregnancy is illegal, misoprostol is used by up to 75% of women who attempt elective self-pregnancy termination [25]. Forty-nine percent of mothers of Brazilian infants with Moebius syndrome report having attempted pregnancy termination using misoprostol in the first trimester [21]. Uterine rupture in first-, second- and third-trimester pregnancies has been reported in women after administration of misoprostol [26–29], with some case series showing an increasing risk of occurrence in women who are undergoing induction of labour in the third trimester and have a uterine scar as a result of previous caesarean section deliveries [26]. Uterine rupture occurs infrequently following misoprostol administration in the first trimester of pregnancy [27].

Serum concentrations of oestrone sulphate of both misoprostol- and control-treated mares in this study remained within the ranges reported for normal pregnancies. Oestrone sulphate concentrations may be used as an indicator of fetal viability [30]. In the pregnant mare, beginning 20 days after breeding, oestrone sulphate concentrations rise to significantly higher concentrations than in nonpregnant mares [31], probably with a contribution from both conceptus [32] and ovarian origins [33]. Serum concentrations have been reported generally to be >30 ng/ml at 80 days of gestation [31], >53 ng/ml at approximately 100 days of gestation [34] and >30 ng/ml in all mares at 120–300 days of gestation [31].

Serum progesterone concentrations also did not change as a result of misoprostol administration. In the mare, progesterone concentration begins to rise as early as 8–12 h after ovulation [35,36], and continues to rise to plateau on Days 6–7 after ovulation [16]. If the mare is not pregnant, serum progesterone concentration then rapidly decreases between Days 13 and 16 [15,37]. If the mare is pregnant, concentrations gradually decrease from Day 12 to 30, followed by a significant rise from Day 32 to 44 and then another rise from Day 44 to 90, after which serum progesterone concentrations decrease as early as Day 120 of gestation [15,16]. Among mares, progesterone concentrations vary widely [15,16]. The mares in this study were between 94 and 140 days of gestation. As expected for mares in

mid- to late gestation, serum progesterone concentrations decreased over the course of the study, both during misoprostol and control treatment; however, concentrations for all mares remained within the range for normal pregnancies, with no apparent effect of treatment.

We conclude that in these 11 mid-gestation pregnant light horse and pony mares, a 5-day gastrointestinal mucosal cytoprotectant oral regimen of misoprostol treatment did not disrupt pregnancy or affect general health or comfort.

Conflicts of interests

No conflicts of interest exist.

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Manufacturer's address

[a]Greenstone Brand Ltd, Peapack, New Jersey, USA.

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