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ORIGINAL ARTICLE

Pharmacokinetics and tolerability of a novel progesterone intravaginal ring in sheep

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Abstract

The objectives of this work were to evaluate the in vitro release and in vivo pharmacokinetics and local tolerability of a novel, segmented ethylene-vinyl acetate (EVA) intravaginal ring (IVR) delivering progesterone (P) in drug-naïve ovariectomized female Dorset crossbred sheep. Following preparation and assessment of in vitro release of P, animals were randomized into one of six treatment groups: group 1 Crinone® 8% gel (90 mg); group 2 Prometrium® 200-mg capsules; group 3 placebo IVR; group 4 progesterone (P) IVR 4 mg/day; group 5 P IVR 8 mg/day; or group 6 P IVR 12 mg/day. Crinone 8% gel and Prometrium capsules were administered once daily for 28 days. IVRs were inserted vaginally on day 1 and remained in place through day 14; a new ring was administered on day 15 and was removed at day 28. Animals underwent daily examinations to confirm ring placement, and vaginal irritation was scored from 0 (none) to 4 (severe). Blood samples were taken at scheduled times for pharmacokinetic analysis. Postmortem examinations performed on all IVR groups included vaginal irritation, macroscopic, and microscopic evaluations, including irritation scoring and histopathology. Intravaginal rings were retained over 28 days in all animals. Clinical observations showed no significant abnormal findings in any group. Pharmacokinetic analysis in animals showed sustained release of P over from days 0 through 14 of ring use. Irritation scores and microscopic assessments were consistent with the IVRs being well tolerated. These results will guide future human clinical studies to ultimately develop an IVR for use in women for the prevention of preterm birth.

Keywords Intravaginal · Progesterone · Pharmacokinetics · Preterm birth

Introduction

Preterm birth (defined as birth before 37 weeks of completed gestation) is the leading cause of neonatal deaths annually causing over 1 million deaths each year, which comprises 27% of 4 million neonatal deaths annually and 3.2 million stillbirths each year [1]. The need for new treatment strategies to reduce preterm birth and infant mortality was summarized recently by the United States Centers for Disease Control and Prevention [2, 3]. Globally, the World Health Organization reports that 15 million preterm births occur each year. The data also suggest the rates of preterm birth are increasing in most countries and that prematurity is the second leading cause of death after pneumonia in children under the age of 5 [4].

Progesterone (P) is a naturally occurring steroid that is secreted predominately by the ovary and placenta. In pregnancy, P helps the uterus grow [5, 6]. In many mammalian species, P

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plays a direct role in uterine quiescence (i.e., preventing labor), and the onset of labor is preceded by a decrease in P plasma concentrations. The role of P in the onset of human labor is less evident [7]. Although the precise mechanism(s) by which the misregulation of P activity leads to cervical shortening and preterm birth (PTB) are not known, there is evidence demonstrating the therapeutic efficacy of delivering supplemental, natural P directly to the cervix to extend gestation. Fonesca et al. demonstrated that daily, vaginal administration of 200-mg capsules of micronized P (Utrogestan®, Prometrium®) in women with a cervical length ≤ 15 mm is associated with a 44% reduction in the rate of PTB < 34 weeks of gestation [8]. Similar findings were reported by DeFranco et al. [9]. Daily administration of micronized P gel (Crinone®) reduces the rate of PTB in women with a normal cervical length between 10 and 20 mm [10]. Further, P was associated with a significant reduction in the rate of respiratory distress syndrome. Neither Crinone or Prometrium are indicated for the treatment of women to prevent preterm birth. A synthetic progesterone product, 17-alpha-hydroxyprogesterone caproate (17-OHPC, Makena®) is indicated to reduce the risk of preterm birth in women with a singleton pregnancy who have a history of singleton spontaneous preterm birth; however, Makena is ineffective in preventing preterm birth in singleton pregnant women with short cervical length (SCL) [11, 12]. To address the unmet needs of women at risk for preterm birth due to SCL, a P-releasing IVR is being developed for this patient population.

Despite the published evidence, medical guidelines, and consensus statements recommending the use of vaginal P for the prevention of preterm birth in women with SCL and singleton pregnancy, there is a paucity of data surrounding the optimal formulation and dosage of P. Systemic absorption of P from gel and capsule products has yet to be compared directly. To address these issues, a 14-day segmented IVR is being developed to deliver a relatively constant dose of P without the need for daily action from the mother.

This report covers the results of the release of P from the EVA-based segmented IVRs under in vitro and in vivo conditions. These rings were designed to release around 4, 8, or 12 mg P/per day over days 2 through 14. Similar IVRs releasing the GnRH agonist leuprolide has been evaluated previously in a small human study over a 3-day time frame [13]. The rings evaluated herein, including placebo IVRs, were administered to drug-naïve ovariectomized female Dorset crossbred sheep for 14 days after which they were removed and a new P-releasing IVR was administered for 14 additional days. For comparison purposes, a group received Crinone 8% gel (approximately 90 mg P) and a second group received Prometrium (200 mg P) capsules intravaginally on a once-daily basis. Data collected included plasma pharmacokinetics of P and microscopic assessment of the uterus, cervix, and vagina.

Materials and methods

Intravaginal rings

Intravaginal rings capable of releasing P were prepared in a manner similar to that described previously [13]. The overall process is shown in Fig. 1. All ring manufacturing took place at QPharma (Malmö, Sweden). The process involved compounding pellets and extrusion of fibers followed by joining of the fibers by heat welding. Blending was accomplished using a Turbula mixer (Model T 10 B, with a 17-L stainless steel mixing vessel, Glenn Mills, Clifton, NJ). The resulting blend was then compounded by hot-melt extrusion using a twin-screw extruder (Pharma 11 twin-screw hot-melt extruder with a Pharma 11 gravimetric feeder) and fed onto a Pharma 11 air-cooled conveyor followed by pelletization using a Pharma 11 Vericut Pelletizor (Thermo Fisher Scientific, Dreieich, Germany). The pellets were formed into fibers by hot-melt extrusion using a 25-mm single-screw extruder (Dr. Collin, Ebersberg, Germany). The resulting fibers were cut using a Dr. Collin in-line cutting station. Cut fibers (or segments) were welded using Automationspartner single station laboratory welder (Ramlösa, Sweden).

IVRs capable of releasing P (EP, micronized, Pfizer, Inc. Kalamazoo, MI) at the desired rates were prepared by using fibers of varying length and drug loading. All IVRs are 57 mm in overall diameter and a cross-sectional diameter of 5 mm. IVRs releasing 4 and 8 mg/day were prepared using EVA (28% vinyl acetate content, Vitaldose®, Celanese Corporation, Boucherville, Canada) with a final drug loading of 27% *w/w*. To create the 4 mg/day ring, the drug-containing segment length was 50 mm with a placebo segment length of 113.5 mm. The 8 mg/day IVR was created with a 100-mm drug-containing segment and a placebo segment of 63.5 mm. The 12 mg/day IVRs were prepared with segments loaded with 36% P (*w*/*w*) with a drug-containing segment of 148.5 mm and a placebo segment of 15 mm. Placebo IVRs were prepared by welding three drug-free segments of 74.0, 74.5, and 15 mm. Each IVR weighs approximately 3 g.

In vitro release of P from IVRs

Before conducting the sheep study, the release rates of P from the three IVR formulations were measured in vitro to determine whether the target release rates had been attained. Release rates were tested using 200 mL 0.5% sodium dodecyl sulfate as a release medium, in shakers at 37 °C. Sampling (2 mL) was conducted at 6 h, days 1–4, 7–11, 14, 15, 18, 21, 22, 25, and 28. Concentrations of P were determined using a validated reverse-phase liquid chromatography method using UV detection. The column used was a Phenomenex Luna C8(2), 150 mm × 3.0 mm, and 5 μm. The guard column used was Phenomenex C8

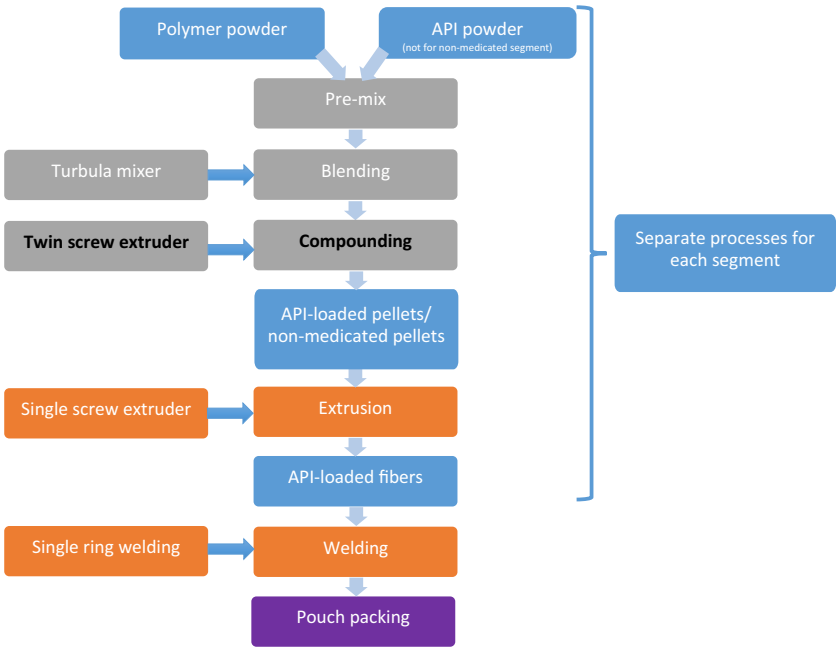

Fig. 1 Schematic showing how to prepare EVA IVRs for release of P

(4 mm × 3 mm). The mobile phase was acetonitrile 45% in purified water (55%), *v*/*v*. The injection volume was 10 μL. P was detected by UV at 245 nm. The standard curve range for P was 0.00625–0.25 mg/mL. Over these concentrations, the curve was linear (correlation coefficient > 0.997). Six IVRs were tested at each dissolution time point.

Sheep study

The purpose of this study was to evaluate the in vivo PK and local tolerability of P releasing and IVRs in drug-naïve ovariectomized female Dorset crossbred sheep and to compare with vaginally administered Crinone 8% gel (1.125 g or 90 mg P; Actavis Pharma, Parsippany, NJ) and Prometrium 200-mg capsules (Solvay Pharmaceuticals, Marietta, GA). Data from the placebo IVR group were also collected as a comparison.

The animal study was conducted by an American Association for Accreditation of Laboratory Animal Care (AAALAC) accredited contract research organization facility (MPI Research, a Charles River Company, Mattawan, MI). The study was conducted in compliance with the US Food and Drug Administration (FDA) Good Laboratory Practices (GLP) Regulations and the US Department of Agriculture (USDA) Animal Welfare Act. A total of 27 experimentally naïve, female, uniparous, Dorset crossbred sheep, approximately 15.5 to 19 months of age at the receipt, were received from Lauwers Lamb, Capac, MI. Animals were identified by implanted microchips and by individual ear tags.

During acclimation, the animals were observed daily with respect to general health and any signs of disease. All animals were given a detailed clinical examination, and body weights were recorded within 3 days of receipt and again prior to the operating procedures. All animals were negative for *Cryptosporidium* and *Giardia* species. *Strongyloides* and *Coccidia* were detected in stool samples from almost all animals. Animals were treated with a single administration of fenbendazole (10 mg/kg orally). Animals weighed 57.5 to 77.0 kg at randomization.

Between 26 and 54 days before the scheduled dosing, all animals underwent a surgical procedure to remove the ovaries, in accordance with the research facility's standard operating procedures. Animals were allowed to recover for 26 to 54 days prior to dosing. During this recovery period, body weight measurements and clinical observations were performed weekly. Ovariectomy surgery was performed successfully in all animals as determined by undetectable levels of endogenous hormones.

During the study, all animals were observed twice daily for morbidity, mortality, injury, and the availability of food and water. Detailed examinations of each animal were performed weekly during the study. These observations included evaluation of the skin, fur, eyes, ears, nose, oral cavity, thorax, abdomen, external genitalia, limbs and feet, respiratory and circulatory effects, autonomic effects such as salivation, and nervous system effects including tremors, convulsions, reactivity to handling, and unusual behavior.

Animals were randomly allocated to one of six treatment groups: group 1 ($n = 3$) Crinone 8% gel; group 2 ($n = 3$) Prometrium 200-mg capsules; group 3 ($n = 3$) placebo IVRs; group 4 ($n = 5$) 4 mg/day IVRs; group 5 ($n = 5$) 8 mg/day IVRs; or group 6 ($n = 5$) 12 mg/day IVRs.

The IVRs were stored at 2 to 8 °C until use and were allowed to warm to room temperature for 30 to 120 mins prior to administration; Crinone 8% gel and Prometrium 200-mg

capsules were maintained at room temperature in accordance with their labels. Both Crinone 8% gel and Prometrium 200-mg capsules were administered on day 1 and every day thereafter ± 2 h through day 28. All IVRs were inserted on day 1 remained in place through day 14; the rings were then removed and a new ring inserted on day 15. The second ring remained in place until day 29 at which time it was removed. Vaginal ring insertion was performed as a clean procedure. The IVR was photographed before being digitally inserted into the cranial vagina using a gloved finger. During the treatment period, animals were digitally examined daily to confirm that the IVR was still in place.

Following the completion of treatment on day 29, the IVRs were removed from each animal and were photographed and stored at 2 to 8 °C before being returned for analysis of residual P content. Residual P in each IVR was performed as follows. Each IVR was cut into 2–3 segments then placed in a 200-mL volumetric flask. Tetrahydrofuran (100 mL at 37 °C) was added to each flask containing the ring segments and shaken at about 180 rpm for 2 h. Following the dissolution of the IVRs, the EVA was precipitated by addition of methanol (90 mL). The resulting solution was passed through a 0.45-μm filter. The filtrate (5 mL) was diluted with 45 mL acetonitrile/H_2O (70:30). The amount of P was determined using a validated HPLC method using UV detection at 220 nm. The theoretical mass balance was calculated by adding the amount of P present in the device after use with the amount released by the IVR (taken from the initial release testing of the IVRs in vitro) and dividing by the theoretical drug content. The amount of P remaining in the IVRs following dosing was determined as a gross check on IVR performance, and results were not intended to be correlated with PK findings.

Pharmacokinetics

Blood samples for determination of the plasma concentrations of P (as applicable) were collected from all animals; animals were not fasted prior to blood collections. Samples from groups 1 through 6 were collected prior to ovariectomy surgery, on day 14 (to confirm successful ovariectomy). In groups 1 and 2, plasma was collected on day 14 pre-dose and again on day 7 pre-dose and at 1, 2, 4, 8, 12, and 24 h post-dose. In group 3, plasma was collected on day 1 pre-dose and at 1, 4, 336, and 672 h post-dose. In groups 4, 5, and 6, plasma was collected prior to insertion of the IVR (0 h), and at 2, 4, 8, 12, 24, 48, 72, 168, 240, 336 h (prior to removal) post-dose.

Blood samples were placed in tubes containing K_2-EDTA and were centrifuged under refrigerated conditions within 60 mins of sample collection. The resulting plasma was stored frozen at − 60 to − 90 °C within 120 mins of sample collection. Plasma samples were shipped on dry ice for analysis (Pyxant Labs, Inc., Colorado Springs, Colorado). Plasma samples were analyzed using a liquid chromatography-mass spectrometry/mass spectrometry method validated according to bioanalytical method guidelines. The standard curve range for P was 0.1–20 ng/mL. Based on quality control samples, accuracy ranged from 96.5–98.0% for P. Precision (% CV) was less than 7.5% for P. The lower limit of quantitation (LLOQ) was 0.1 ng/mL with an upper limit of quantitation of 20 ng/mL. Concentrations below the LLOQ were set to zero for PK analyses.

Standard noncompartmental PK analysis methods were used. The area under the concentration-time curve (AUC) values were estimated by the trapezoidal rule. The C_{avg} (defined as average plasma concentration over the entire dosing interval, calculated as AUC_{TAU}/dosing interval where TAU is the dosing interval for steady-state data) was determined following administration of Crinone 8% gel and Prometrium 200-mg capsules ($AUC_{0–24\ h}$/24 h) or IVR administration calculated as $AUC_{0–336\ h}$/336 h.

Tolerability: description of assessments, grading scale

The external vagina (the vulva and the externally visible portion of the vestibule) of all animals was examined prior to administration of all test articles and daily examinations were conducted on days 2 through 29, prior to the daily ring checks or product administration. The external vagina was observed for gross signs of irritation (i.e., erythema and edema) and any other signs of local or systemic effect. Irritation was scored based on the Draize scale [14]; erythema and edema formation were rated on a scale of 0 (none) to 4 (severe). The same scales were used at necropsy on day 29 to score irritation of the internal vagina (the portion not visible during in-life assessments); any other signs of local or systemic effects were also recorded.

Necropsy

On day 29, following external vaginal irritation scoring and ring removal, animals in all groups were euthanized. At necropsy, a macroscopic examination of the reproductive organs and surrounding tissues was performed and the uterus, cervix, and vagina were collected and fixed in 10% neutral buffered formalin. Microscopic examination of reproductive tissues was conducted routinely processed hematoxylin- and eosin-stained slides by a board-certified veterinary pathologist (J.D.V).

Vaginal irritation was scored based on the rabbit vaginal irritation method described by Eckstein et al. [15]. For each animal, 3 vaginal regions including the portion adjacent to the cervix (cranial), the middle portion (mid), and the portion at the level of the urethra (uro) were scored separately for 4 parameters (epithelial damage, vascular congestion, edema,

and leukocyte infiltration) with each parameter receiving a score of 0 (normal) to 4 (marked). An overall vaginal irritation score was calculated for each vaginal region for each group by taking the sum of scores for all 4 parameters per site, dividing by the number of animals, and subtracting the average for the placebo control group from a companion study under similar conditions. Therefore, the overall vaginal irritation score ranged from 0 to a maximum of 16.

Statistical analyses

Statistical analysis of data was limited to calculation of descriptive statistics, including means, standard deviations (SD), group size for each group and time period (continuous endpoints), and either medians or incident counts for each group and time period (categorical endpoints).

Results

Release rates of P over the 14-day test period are characterized by the rate over day 1, from day 2 to day 14, and the rate on day 14. Table 1 shows the data collected in this manner from the different P-releasing IVRs. The release rate of P from day 2 through 14 were close to the target release rates of 4 mg/day (3.8 ± 1.3 mg/day), 8 mg/day (7.4 ± 3.3 mg/day), or 12 mg/day (11.5 ± 2.8 mg/day). The in vitro release profile is typical of a matrix-type delivery system with a relatively rapid release of drug followed by a period of slower release which can be seen in Fig. 2.

Following administration of the IVRs, daily vaginal ring checks confirmed the IVRs remained in place until the time of removal on day 15 and day 29. All rings remained in place for their appropriate duration with the exception of one animal in group 5 (8 mg/day) on day 16 when it was apparently expelled and could not be located. Therefore, a new ring was inserted in this animal on day 16. Additionally, on day 21, a ring from one animal in group 6 (12 mg/day) was found in the bedding. The ring was visually inspected for damage, cleaned with lukewarm water, dried, and reinserted.

The plasma concentration versus time curves for P for groups 1 and 2 indicate a somewhat rapid absorption process (T_{max} of 2 h) and first-order elimination with a tenfold range of concentrations during a steady-state dosing interval (Fig. 3). The PK parameters on day 14 of once-daily vaginal administration of Crinone 8% gel and Prometrium 200-mg capsules are summarized in Table 2. Despite approximately half of the dose administered as Crinone 8% gel compared with Prometrium (90 mg vs. 200 mg), C_{max}, and $AUC_{0–24\ h}$ were substantially higher indicating a greater rate and extent of absorption from Crinone.

The concentration over time for the 4, 8, and 12 mg/day IVR groups (Fig. 4) generally showed a sustained release profile. This indicates a slow release and absorption process, the rate-determining step in the prolonged 14-day profile. Table 3 shows the PK parameters following administration on day 1 and followed for 14 days. In general, the PK parameters from the three different IVR increased with increasing release rate. While not directly comparable, the PK parameters from the 12 mg/day IVRs were most similar to those from Crinone 8% vaginal gel. Following removal of the IVRs on day 29 (the second ring administered), the rings were analyzed for residual P. The amounts released were calculated by adding the amount of P measured in the rings after 14 days of use in sheep to the cumulative amount released over 14 days under in vitro dissolution, and then dividing by the content as measured by assay. Doing so showed that all rings were within ± 10% of the mass balance. There was consistency between in vitro dissolution and in vivo release as well. For instance, the 8 mg/day IVRs had an initial average loading of 514 mg P; following 14 days of release in the animals, the residual mean amount of P remaining was 387 ± 34 mg ($n = 5$) or 130 mg P released over 14 days. In vitro, the cumulative amount of P release over 14 days was 139 mg. Similar results were found for IVRs releasing P at 4 and 12 mg/day.

Animal evaluations conducted during the treatment period were unremarkable for abnormal physical findings. Enlarged udders were observed in several animals (including some animals with enlargement prior to administration of study treatment), but since all animals had a history of lactation, these observations were not considered to be related to the study treatment. One group 2 animal was observed with the red coloration of the vulva during week 4, which correlated with vaginal irritation findings (see below). A clear vaginal discharge was observed in two animals from group 4 during week 3 and weeks 3 and 4, respectively. Discharge findings are common during studies with the intravaginal route.

Evaluations of external vaginal irritation performed on days 1 through 29 of the treatment period comprised assessments of erythema and edema on a scale of 0 to 4 (Online

Table 1 Release rates (mg/day) from the 4 mg/day, 8 mg/day, and 12 mg/day P IVRs

Test time point(s)	4 mg/day IVR (mg/day)	8 mg/day IVR (mg/day)	12 mg/day IVR (mg/day)
0–24 h	20.5 ± 2.2[a]	38.4 ± 3.3	60.5 ± 2.5
2–14 day	3.8 ± 1.3	7.4 ± 2.4	11.5 ± 2.8
14 day	2.3 ± 1.5	4.7 ± 1.9	7.3 ± 3.8

[a] All data are means ± SD ($n = 6$)

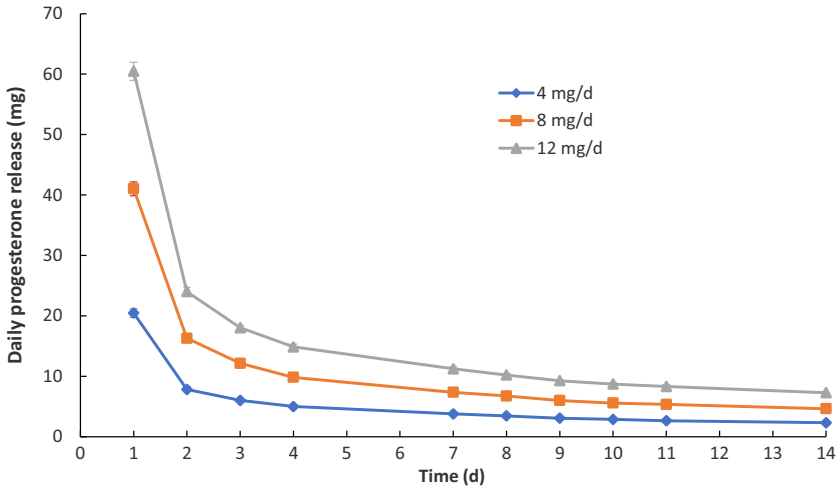

Fig. 2 In vitro release of P from IVRs (4 mg/day, 8 mg/day, and 12 mg/day). Data are means ± SD ($n = 6$)

Resource Table 1). The number of incidences of very slight erythema and eschar was slightly elevated for animals administered the highest IVR dose (12 mg/day); however, the irritation was limited to two of the five animals in the treatment group. In all other treatment groups, very slight erythema was observed in only one animal on two occasions or in two animals on one occasion. Very slight edema was observed on no more than one occasion/animal in the Crinone 8% gel and placebo and 12 mg/day IVR-treated groups. All external vaginal irritation findings were transient and were no longer present by study termination. The findings were most commonly observed after the first insertion of the IVR-treated groups and during the first 14 days of study for the Crinone 8% gel-treated group.

Incidence counts of internal erythema and edema scores are also summarized in Online Resource Table 1. The number of incidences of very slight and well-defined erythema was slightly elevated for animals administered the low dose IVR (4 mg/day). Very slight erythema was observed in 4 of 5 animals in the mid dose of IVR (8 mg/day). The high-dose IVR group (12 mg/day) had the lowest number of incidences (2 of 5 animals) with one animal observed with very slight and one animal observed with well-defined erythema. In all other treatment groups, no erythema was observed in any animal. Due to the lack of a dose response and lack of a microscopic correlate, these findings were not considered to be IVR related.

IVR-related microscopic observations are summarized in Online Resource Table 2. The uterus, cervix, and vagina were generally small and inactive in all animals, which were considered a normal feature in ovariectomized animals. There was a minimal to mild mononuclear cell infiltration present within the vagina of all animals including the placebo controls, which was often accompanied by a minimal to mild neutrophilic infiltration. Minimal focal epithelial degeneration was present within the vagina of two individual animals, including placebo controls. Due to the lack of a dose response and similarities across the groups including the placebo IVRs, these changes were not considered to be IVR related.

Assessment of the vaginal irritation index according to the method of Eckstein (Table 4) showed minimal irritation in all

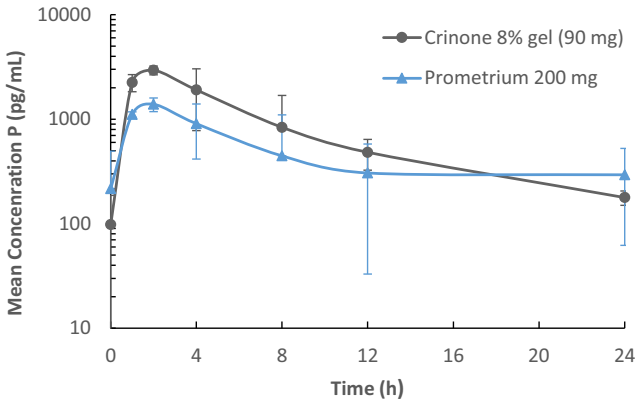

Fig. 3 Plasma concentration-time profiles of P on day 14 following once-daily dosing of Crinone 8% gel (90 mg P) and Prometrium 200-mg capsules. Data are mean ± SD ($n = 3$)

Table 2 PK parameters from Crinone 8% gel (90 mg) and Prometrium 200-mg capsules

PK parameter		Group 1 90 mg P[a]	Group 2 200 mg P[b]
C_{max}	(pg/mL)	3020 ± 140	1390 ± 206
$AUC_{0–24\ h}$	(h*pg/mL)	20,700 ± 1640	12,000 ± 4090
C_{AVG}	(pg/mL)	863 ± 68.5[c]	501 ± 170
T_{max}	(h)	2 (2–4)[d]	2 (2–2)

[a] 90-mg dose of P is approximately 1.5 mg/kg based on a sheep of 60 kg

[b] 200-mg dose of P is approximately 3.3 mg/kg based on a sheep of 60 kg

[c] $C_{AVG} = AUC_{0–24\ h}/24$ h

[d] Median (minimum–maximum), median value only reported if actual collection interval

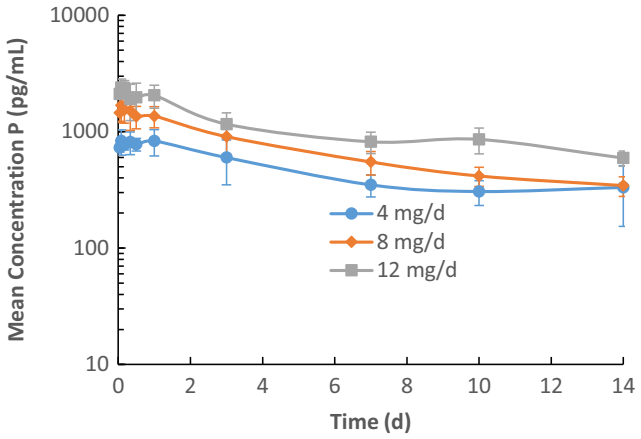

Fig. 4 Plasma concentration-time profiles of P from day 0 through day 14 following a single administration of IVRs releasing P at 4 mg/day, 8 mg/day, and 12 mg/day. Data are mean ± SD ($n = 5$)

treatment groups, with irritation scores typically highest in the cranial portion of the vagina where the IVR was placed. Median scores in all groups were within the minimal irritation category (defined as scores of 1–4).

Discussion

A short cervix at mid-pregnancy is the most reliable predictor of preterm birth of a newborn. Children who are born preterm have a greater risk of chronic and life-threatening conditions at all stages of life. In infancy, these include respiratory distress syndrome, necrotizing enterocolitis, intraventricular hemorrhage, retinopathy of prematurity, and sepsis. In early childhood, they are at an increased risk for developmental delay, behavior and learning problems, and asthma. In adulthood, those who are born prematurely have an increased risk of arterial hypertension, diabetes mellitus, cardiovascular disease, and stroke [16–19]. Over a decade of evidence supports the use of vaginally administered P for the prevention of preterm birth in women having a short cervix at mid-pregnancy [8, 9, 20–22], yet there is still no FDA-approved progesterone product for this indication.

The development of new interventions that effectively extend the term of gestation, thus reducing the incidence of preterm birth remains a significant unmet need. To advance the standard of care for women at risk for preterm birth, the goal of this work is to couple the current, unoptimized, standard of medical care with an intravaginal ring technology to improve the benefits of vaginal progesterone therapy. To reach this goal, the IVRs should deliver a relatively constant and optimized dose of P over a 14-day therapeutic window, without the need for daily action from the mother.

The IVRs used in this study are composed of EVA, a common polymeric excipient used in many applications including NuvaRing, a 3-week contraceptive delivery system [23–25]. NuvaRing is a reservoir device prepared by coaxial extrusion and welded together to form a torus-shaped device [26]. In contrast, the rings evaluated in this study, while also prepared with EVA, are formulated as matrix devices. As a result, the release kinetics are consistent with matrix-type devices in that release is linear against the square root of time (data not shown). The segmented ring offers a few advantages. First, the loading of segments can be optimized within a narrow range of concentrations (27 to 36%). This range, in 28% vinyl acetate content EVA, ensures a stable system that avoids being at or around a transition point between fully dispersed drug in polymer and crystalline drug particles dispersed in the polymer. The second advantage is the ability to easily change release rates through variations in segment length. As a result, preparation of clinical supplies for dose-ranging studies is straightforward.

There have been a number of IVR formulations designed to release P over an extended period of time. An early example of a P-releasing IVR was a silicone reservoir device to be used by post-partum, lactating women for birth control [27]. These IVRs were designed to release about 5 mg per day for 90 days. More recently, another silicone IVR was developed and commercialized (Progering®, Grunenthal Laboratories) in several South American countries. Progering releases P at about 10 mg per day for 90 days [28–30]. Vaginal rings releasing P have also been investigated for luteal support during in vitro fertilization [31–35] although none have been approved for use by regulators.

Table 3 Pharmacokinetic parameters of P from intravaginal rings

PK parameter		Group 4 4 mg/day	Group 5 8 mg/day	Group 6 12 mg/day
C_{max}	(pg/mL)	969 ± 145	1820 ± 469	2520 ± 432
$AUC_{0–336\ h}$	(pg*h/mL)	153,000 ± 38,900	229,000 ± 40,700	350,000 ± 73,900
C_{AVG}	(pg/mL)	455 ± 116[a]	682 ± 121	1040 ± 220
T_{max}	(h)	12 (1–72)[b]	2 (1–8)	4 (2–8)

[a] $C_{AVG} = AUC_{0–14\ days}/14$ days

[b] Median (minimum–maximum), median value only reported if actual collection interval

Table 4 Collective group mean IVR vaginal irritation scores

Vaginal location	Treatment			
	Placebo (group 3) $n = 3$	4 mg/day (group 4) $n = 5$	8 mg/day (group 5) $n = 5$	12 mg/day (group 6) $n = 5$
Cranial	1.67	− 0.47	− 0.67	− 0.47
Mid	1.00	0.20	0.00	0.20
Uro	1.33	− 0.13	− 0.33	− 0.53

The present study was conducted to prepare IVRs that were capable of releasing P at varying rates. Both comparator products evaluated (Crinone gel and Prometrium capsules) in this study have been shown to clinically reduce the frequency of preterm birth (spontaneous delivery before 34 weeks) in women with a short cervix (< 15 mm or ≤ 25 mm) [8, 36]. Crinone 8% gel was evaluated by the US FDA for licensure but was ultimately rejected following a more robust statistical analysis than initially used by the sponsor [37]. Despite this failure, it is important to understand the pharmacokinetics of both products to provide insights into a target dose from a P-releasing IVR. Such a comparison is qualitative due to the difference in dosing regimens between the once-daily administration compared with biweekly administration. A comparison of the C_{AVG} of Crinone 8% gel and the three IVRs tested indicate that the 8 mg/day and 12 mg/day P-releasing IVRs gave values similar to that observed from Crinone. The advantage of the IVR dosage form over Crinone is the significantly increased dosing interval which should improve patient compliance and improve therapeutic outcomes.

Of interest are the differences noted between the PK parameters between Crinone 8% gel (90 mg P) and Prometrium 200-mg capsules. P was absorbed more effectively from the lower dose gel compared with the capsules. Prometrium capsules were designed for oral administration rather than the vaginal administration. It is unsurprising then that the dissolution and absorption of P following vaginal dosing of Prometrium are different from a vaginal gel designed to deliver P to the vagina.

The rings were in general well tolerated in the sheep vagina over the time frame tested. The rings are basically composed of two compounds, P and EVA. EVA is generally well tolerated as a biomaterial and P is a naturally occurring hormone. The most likely source of local irritation leading to a tissue response is probably the simple physical presence of the IVR in the vagina.

In conclusion, the data obtained from this study demonstrate that these segmented EVA IVRs are capable of sustained in vivo release of P at varying rates over a 14-day period. The IVRs showed little signs of vaginal irritation or mucosal breaches. These results support the conclusion that the EVA P-releasing IVRs are suitable for evaluation in phase 1 clinical study in women.

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