Actions of progestins for the inhibition of cervical ripening and uterine contractions to prevent preterm birth

R.-J. Kuon[1], R.E. Garfield[2]

[1]*Department of Obstetrics and Gynecology, University of Heidelberg, 69115 Heidelberg, Germany.*
[2]*Department of Obstetrics and Gynecology, St. Joseph's Hospital and Medical Center, Phoenix, Arizona 85013, USA.*

Correspondence at: Robert.Garfield@DignityHealth.org

Abstract

The importance of progesterone (P4) for maintenance of pregnancy, its role in cervical ripening and uterine contractions is at least partly established and therefore, not surprisingly, the basis for the concept to use P4 as a treatment for preterm birth. Due to the complexity of the condition of preterm birth there are still questions concerning the optimal population that might benefit, timing of treatment, dosage, vehicle and route of administration. Recently vaginal P4 and intramuscular 17-alpha-hydroxyprogesterone caproate (17P) have been used to prevent preterm birth in patients with a high risk for early delivery.
The aim of this study was to assess cervical changes throughout pregnancy in rats and the timing of term and preterm delivery after various progestin treatments given by different routes and vehicles in hope of identifying better treatment regimens.
This paper presents results that suggest that there are better routes of treatment than the vaginal route (e.g. topical), that the vehicle used in many of the clinical studies (Replens®) is not appropriate due to a low release of the steroid and consequently low uptake of P4, and that inhibition of birth is primarily due to inhibition of uterine contractility that can be achieved by supplementation of P4 but not with 17P.

Key words: **17-alpha-hydroxyprogesterone, cervical ripening, preterm birth, progesterone, uterine contractions.**

Introduction

Preterm birth is the major challenge in obstetrics today. Despite all efforts and intensive research, the rate of preterm birth has risen in the last years and varies from about 5% to 11% in Europe (Euro-Peristat Project, 2008). Although only a small number of all preterm births occur before 34 weeks (approximately one sixth), these infants are especially endangered and are responsible for the majority of morbidity, mortality and costs (Werner et al., 2011). There are even higher rates of preterm birth observed in the USA, where the problem escalated in the last 25 years with an increase of the preterm birth rate of 36% (Martin et al., 2007). The development of effective therapies to prevent or reduce the occurrence of this difficult medical condition depends on the understanding of the circumstances that initiate labor. Many biochemical and functional changes occur in the cervical connective tissue during gestation, which are summarized in the term cervical ripening. It is an active biochemical process with similarities to an inflammatorylike reaction and occurs independent of uterine contractions (Liggins, 1981; Garfield et al., 1998). Arpad Csapo was a pioneer in characterizing P4 as a key player for the maintenance of pregnancy (Csapo, 1956). Csapo's observations were mainly derived from animal studies on rabbits and rodents where, in contrast to primates and humans, a sharp P4 withdrawal occurs before the onset of labor and delivery. The biochemical and molecular actions of P4 in this specific context were illuminated by several studies. Undoubtedly, if P4 action is blocked, the cascade of cervical ripening will lead to clinical, biochemical and morphological changes (Chwalisz and Garfield, 1994). Despite the findings and the progress the exact mechanism how P4 exerts its effects on the uterus is still unrevealed. Nevertheless, supported by experimental results from animal and in vitro

studies, there is growing evidence also from large randomized placebo-controlled clinical trials that a P4 supplementation reduces the rate of preterm birth in a certain subpopulation of patients that might be identified in an asymptomatic state in midgestation by vaginal ultrasound examination and by measuring the cervical length (da Fonseca et al., 2007; Hassan et al., 2011; Romero et al., 2012).

A better understanding of the mechanisms of preterm labor, a method to diagnose endangered women and the renewed appreciation of the role of P4 in the prevention of preterm labor are key factors in the concept of pharmacological prevention of preterm birth. Nevertheless, important questions concerning the exact mechanisms of action, optimal progestin formulation, dosage, route of administration and timing of therapy are still only partly answered. We therefore performed in vitro and in vivo studies in the rat, focusing on the type of progestin, the route of administration and the vehicle used for vaginal and topical administration using rats that deliver at term withouth prior treatment with progestins. This is a well recognized bioassay of progestin and antiprogestin potency that has been used reliably in many studies. Time of delivery was determined and cervical changes were assessed by an optical method using an endoscopic camera and by measurements of light-induced fluorescence with an instrument called collascope.

Materials and Methods

Timed-pregnant Sprague-Dawley rats (N = 6/ group) were treated from day 13 of pregnancy until delivery. Single daily treatments were performed at 8 a.m. and twice a day treatments at 8 a.m. and 8 p.m. Treatments: Injections (subcutaneous (s.c.), daily): vehicle: sesame oil; P4 (4 mg); R5020 (2 mg); 17P (10 mg); vaginal (bid): vehicle: Replens® (base for Crinone®, Columbia Laboratories) ; P4 (15 mg, Crinone®); R5020 (1 mg); oral (bid): vehicle: sesame oil or H_2O; P4 (15 mg); topical (bid): vehicle: Replens®, sesame oil or fish oil; P4 (15 mg). The control rats were treated with the appropriate vehicles. The antiprogestin, RU-486 (3 mg in 0.2 ml sesame oil), was injected s.c. once on day 16 of gestation.

For the measurements with the collascope and the endoscopic camera the animals were anaesthetized. The amount of cervical collagen (assessed by measurement of the autofluorescent properties of cross-linked collagen with an instrument, termed collascope (Reproductive Research Technologies, Houston, TX, USA) and changes of the surface area of the cervix (calculated from digitized photographs by morphometric methods using the software ImageJ 1.43) were evaluated in vivo every second day starting on day 13 of pregnancy and on postpartum days 3 and 5. Student's t-test was used to compare the results of a treatment group to its specific control group at any time in gestation and postpartum and also to determine the differences in delivery times. A two-tailed probability value of $P < 0.05$ was considered statistically significant.

For more details see the material and method section in the two publications by Kuon et al. (Kuon et al., 2010; 2011). All procedures were approved by the Animal Care and Use Committee of the St. Joseph's Hospital and Medical Center in Phoenix.

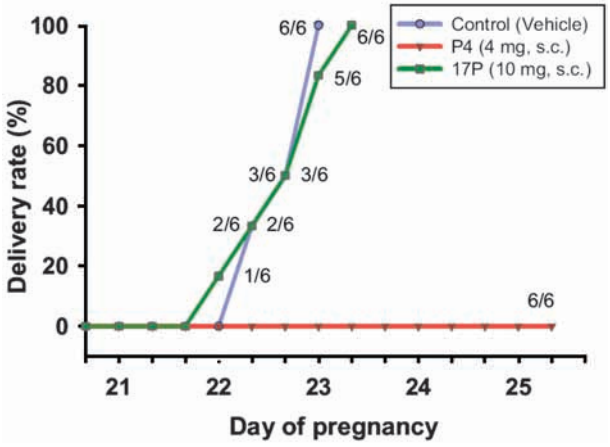

Fig. 1. — The percentage of animals (N = 6/ group) that delivered vs. day of pregnancy after various treatments. Delivery times after daily treatment (s.c.) with vehicle, P4 and 17P. Note that injections of P4 completely blocked delivery, whereas 17P had no effect on delaying term delivery (Kuon et al., 2010).

Results

Effects of treatment with P4

Subcutaneous and topical P4 with vehicle sesame oil, partially, or fish oil, completely blocked delivery. Vaginal, oral and topical P4 in Replens® failed to inhibit delivery (Fig. 1, 6).

Light-induced fluorescence (LIF) is significantly ($P < 0.05$) higher in the P4 injection group and increases in surface area (SA) of the cervix are lower correspondingly compared with vehicle controls for any day of gestation (Fig. 3A, 3B). There is no difference ($P > 0.05$) between day 25 (delayed delivery) in the P4 injection group (Fig. 1) compared to control animals at day 21 of gestation. LIF and SA before treatment at day 13 show no significant

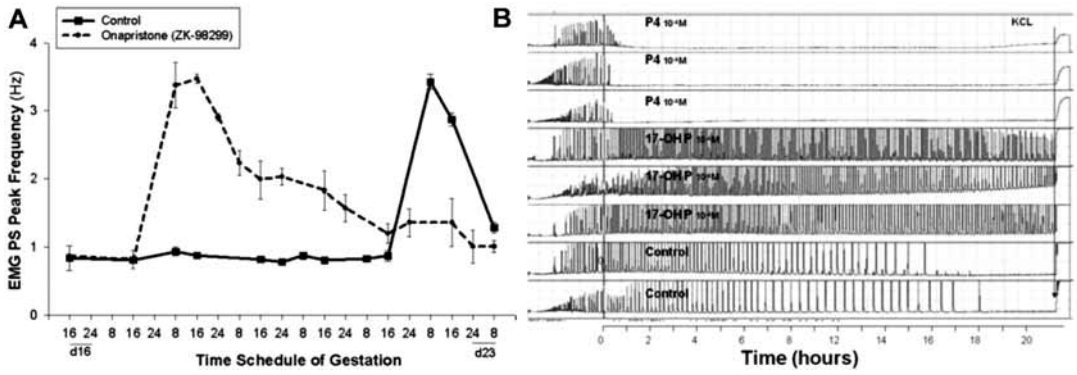

Fig. 2. — **A.** EMG activity in animals delivering at term and preterm after onapristone. Treatment with onapristone (ZK-98299) on day 16 of pregnancy induces preterm delivery and an increase of the electromyographic power spectrum (PS) peak frequency in rats (Modified from figures in Shi et al., 2008) ; **B.** The effects of P4 (top three traces) and 17P (= 17-OHP); traces 4–6 from top) on myometrial contractility. Potassium chloride was added to the baths just prior to termination of the experiment. Note the inhibition of myometrial contractility with P4, but not with 17P (Ruddock et al., 2008).

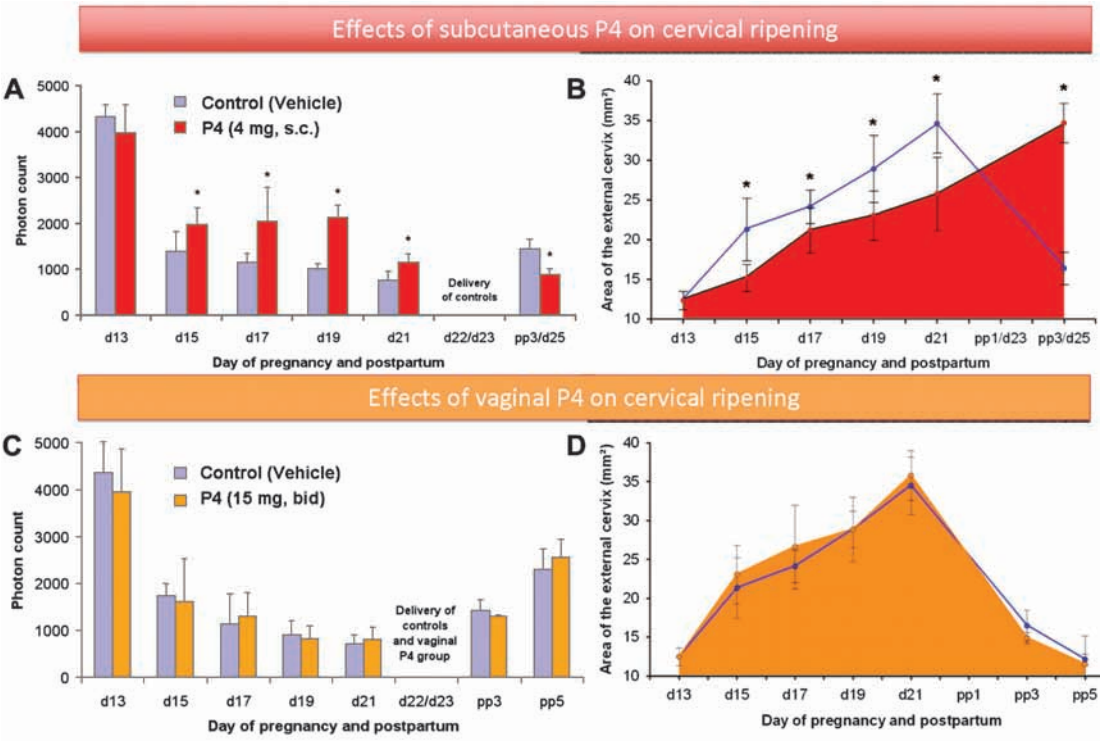

Fig. 3. — **A + C.** Bar graphs showing means ± SD of cervical light-induced fluorescence (LIF) obtained in vivo from pregnant rats at different days of pregnancy and postpartum (N = 6/ group) treated with P4 or vehicle. Figure 3A: Daily treatment with vehicle (controls) or P4 (4 mg, s.c.). Note that delivery is inhibited in the treatment group. Figure 3C: Twice a day treatment with vehicle (controls) or vaginal P4 (15 mg bid). Note that no significant differences are observed at any time between controls vs. treated animals (From Kuon et al., 2010) ; **B + D.** Figure showing surface area (SA) of cervix (means ± SD of the surface area of the cervix) obtained in vivo from pregnant rats (N = 6/ group) at different days of pregnancy and postpartum treated with P4 or vehicle (same treatment regime as for Figure 3 A + C) (From Kuon et al., 2011).
Asterisks indicate $P < 0.05$ compared with controls.

differences ($P > 0.05$) between the treatment and the control group and this is similar for all treatment groups mentioned with other treatments (Figs. 3, 4).

There are no significant differences ($P > 0.05$) between the vaginal P4 group and vehicle controls at any time in gestation (Fig. 3C, 3D).

Effects of treatment with 17P

17P failed to inhibit delivery in rats (Fig. 1, 6). LIF is significantly higher ($P < 0.05$) and increases in SA are lower in the 17P treated group (until day 19 only) compared with vehicle controls (Fig. 4).

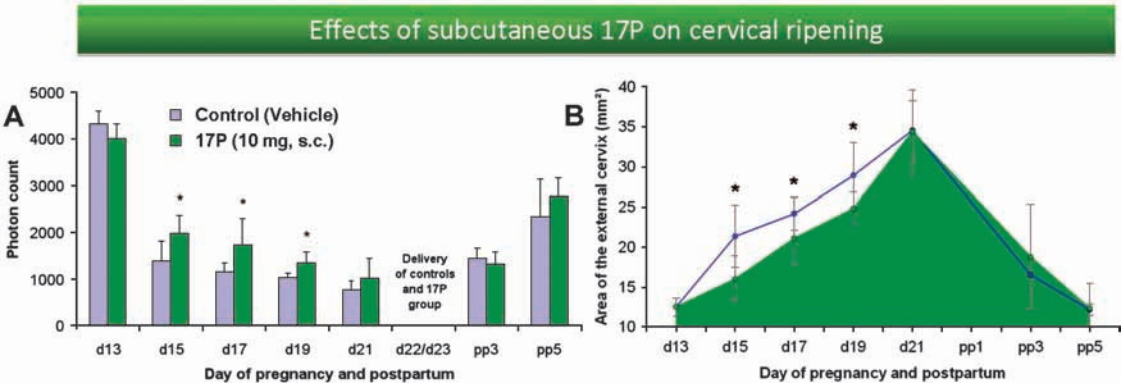

Fig. 4. — Daily treatment of pregnant rats (N = 6/ group) with vehicle (controls) or 17P (10 mg, s.c.). Note that significant differences are only observed until day 19 of gestation (From Kuon et al., 2010; 2011).

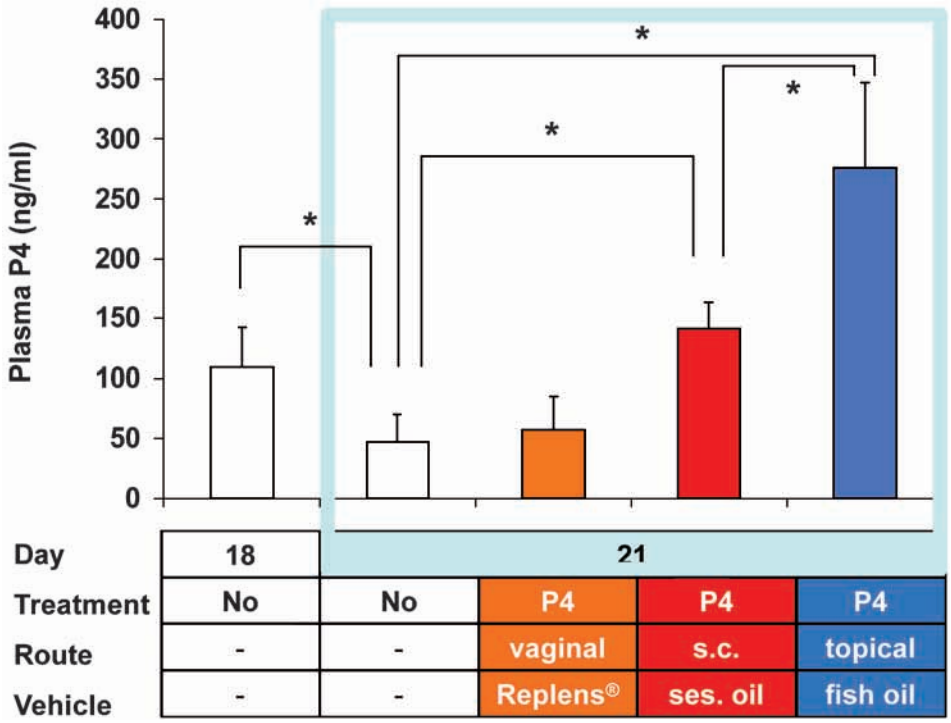

Fig. 5. — Plasma P4 levels in pregnant rats (N = 6/ group) on day 18 and 21 without any treatment (control) and on day 21 after treatment from day 18 until delivery with vaginal P4 (15 mg, bid), s.c. P4 (4 mg), or topical P4 in fish oil (15 mg, bid). Asterisks indicate $P < 0.05$ compared with controls. Note the physiological P4 withdrawal from day 18–21 in untreated rats, which is prevented by s.c. and topical P4, but not by vaginal P4 (From Kuon et al., 2010).

Effects of treatment with R5020

Subcutaneous injections (s.c.) and vaginal R5020 completely blocked delivery (Fig. 6).

Effects of treatment with RU-486

RU-486 treatment increases the SA and LIF are lower ($P < 0.05$) during preterm delivery (rats were delivering 24-48 hours after injection) compared to controls (results not shown).

Comment

As shown in this study progestins have the ability to delay cervical ripening and delivery in term pregnant rats. These effects depend critically on the choice of the progestin, vehicle and the route of administration. An optical evaluation of the exterior of the cervix and the use of light-induced fluorescence (LIF) of cross-linked collagen with an instrument called collascope can be useful to assess quantitative changes in cervical ripening in vivo. Throughout

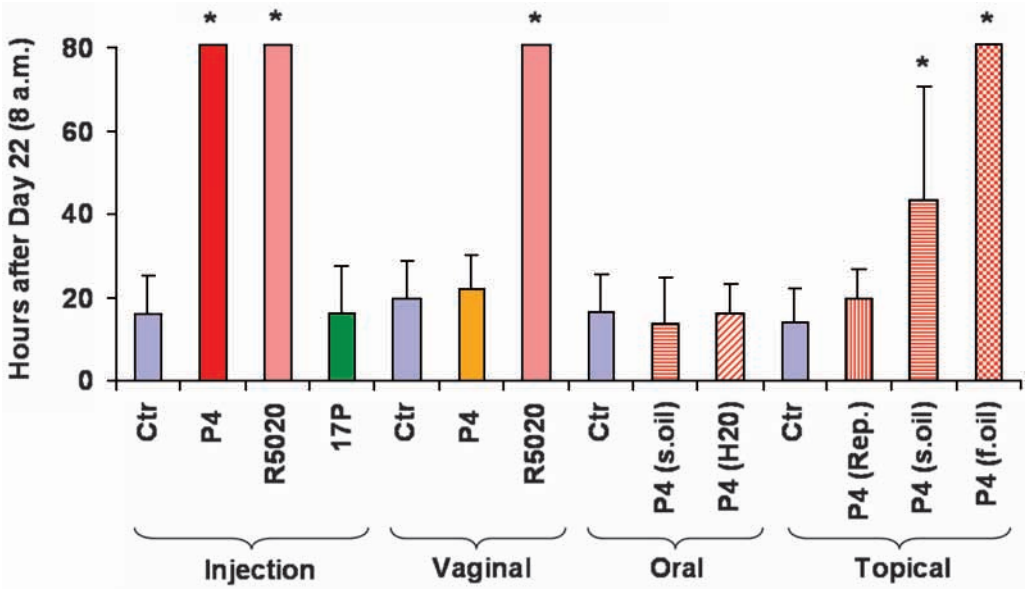

Fig. 6. — Time of delivery (= hours after 8 a.m. of day 22 of gestation) of pregnant rats (N = 6/ group) treated (daily from day 13 of gestation) with vehicles (Ctr) and various progestins by different routes of administration – injections (s.c.; daily): vehicle: sesame oil; P4 (4 mg); R5020 (2 mg); 17P (10 mg); vaginal (bid): vehicle: Replens®; P4 (15 mg, Crinone®); R5020 (1 mg); oral (bid): vehicle: sesame oil or H_2O; P4 (15 mg); topical (bid): vehicle: Replens®, sesame oil or fish oil; P4 (15 mg). Asterisks indicate $P < 0.05$ compared with controls (From Kuon et al., 2010).

gestation the collascope indicates a decreasing photon count which describes the remodelling of the extracellular matrix including a decrease in collagen concentration and switch from insoluble to more soluble collagen (Kuon et al., 2010). This decrease of collagen results in the softening of the cervix which is assessed by the endoscopic camera with an increase in SA of the cervix as a consequence. We hypothesize that the increase of the surface area of the external cervix during cervical ripening is due to the rearrangement of the extracellular matrix, the dilatation of blood vessels, the increase of hyaluronan and the influx of water causing the cervical tissue to swell. As anticipated in the postpartum period the LIF increases progressively indicating a reversal of ripening, whereas the SA of the cervix decreases. As the decrease of LIF and the increase in SA of the cervix are decelerated in the parenteral (s.c. injection) P4 (Fig. 3A, 3B) and 17P (Fig. 4A, 4B) group we conclude that these treatments delay cervical ripening but do not entirely prevent it, indicating the involvement of other control pathways. Vaginal P4, even at 7.5 X the parenteral dose, does neither inhibit ripening, as indicated in this study by changes in the LIF or the SA of the cervix, nor delivery possibly because of reduced uptake (Fig. 3C, 3D). P4, but not 17P, inhibits delivery and may be more effective for treatment of preterm labor (Fig. 1, 6). However this depends crucially on the route of administration and the vehicle, as only s.c. and topical P4 in sesame oil (partially) and fish oil (completely), but not vaginal, oral or topical P4 in Replens® blocked delivery (Fig. 6). As the cervix manages to ripen also in the parenteral P4 treated group at the end of gestation we conclude that the block of delivery is not due to an unripe cervix but must be due to an inhibition of uterine contractions.

The present study does demonstrate significant differences in cervical ripening in early pregnancy with some progestin treatments and this may correspond to differences in cervical length estimated in some of the clinical studies of progestin treatment (Facchinetti et al., 2007; O'Brien et al., 2009).

Current studies for prevention of preterm birth with P4

P4 has long been considered as a candidate to regulate uterine contractility and cervical function and consequently the onset and progression of labor. Early studies also discussed the potential benefit of 17P for the treatment or prevention of preterm labor (Johnson et al., 1975; Keirse, 1990). Several clinical studies investigated in the last few years the effects of a treatment with intramuscular 17P and P4 given orally and vaginally on the reduction of preterm birth and cervical changes (Durnwald et al., 2009; Facchinetti et al., 2007; Fonseca et al., 2003, 2007; de Franco et al., 2007; Hassan et al., 2011; O' Brien et al., 2007, 2009; Rai et al., 2009). These studies and their conclusions raise questions about the ability of P4 and 17P to inhibit preterm birth and also

were difficult to compare, as the treatments and study populations included were significantly different.

As recommended in a current report of the American College of Obstetrics and Gynecology (ACOG) Committee and the Society for Maternal Fetal Medicine (SMFM), P4 should only be used for the prevention of preterm delivery in a "singleton pregnancy with a prior spontaneous preterm birth due to spontaneous preterm labor or premature rupture of membranes" (Society for Maternal Fetal Medicine Publications Committee. ACOG Committee Opinion number 419 October 2008 (replaces no. 291, 2008)). The Committee pointed out that the ideal P4 formulation still is unidentified, as well as the dosage and route of administration.

In a systematic review, utilizing individual patient data of five trials using vaginal P4 for prevention of preterm birth in women with an asymptomatic sonographic short cervix, Romero et al.(2012) tried to address this problem and concluded that the treatment was associated with a significant reduction in the rate of preterm birth before 28 (number needed to treat (NNT) 18), 33 and 35 weeks and also an improved neonatal outcome. The study showed a 42% reduction of the rate of preterm birth before 33 weeks of gestation in singleton pregnancies that received vaginal P4 (Prochieve®, 8% micronized progesterone in Replens base, Columbia Laboratories---the same product we used in this study for vaginal P4), which was the primary outcome (NNT 11). A significant reduction was shown for women with a previous history of preterm birth as well as for women without. The study also pointed out that there is no difference in efficacy of 90-100 mg vaginal P4 administered as a gel compared to 200 mg P4 capsules. However in twin gestations vaginal P4 failed to reduce the rate of preterm birth significantly before gestational week 33. As the optimal cut-off of a short cervix is still not determined, Romero et al. (2012) chose a cervical length below 25 mm in midtrimester, as this length represents the 10th percentile for the cervical length in this period of gestation. Romero also suggested P4 supplementation as an alternative therapy to cerclage in women with a singleton gestation, previous spontaneous preterm birth and a short cervix < 25 mm, as the cerclage reduces preterm birth before 35 weeks by 30 % (Berghella et al., 2011) whereas the non-invasive P4 shows a reduction before 33 weeks by 46% (Berghella et al., 2011; Romero et al., 2012). Despite some controversial results, the U.S. Food and Drug Administration (FDA) in 2011 approved Makena® (17 alpha hydroxyprogesterone caproate) to reduce the risk of preterm delivery before 37 weeks of pregnancy, in pregnant women with a history of at least one spontaneous preterm birth. In February 2012, due to inadequate data, the FDA disapproved the P4 vaginal gel Crinone® for the reduction of risk of preterm birth in women with short uterine cervical length at the midtrimester of pregnancy, saying that "Crinone® was not more effective than the placebo". The FDA panel especially was sceptical about foreign data as analyses indicated that P4 gel was not associated with a reduction in preterm birth in the U.S. subpopulation at any gestational age. Our studies reviewed in this paper predicted the outcome of the FDA trial for Crinone®. However the members of the advisory board of the FDA agreed at least that the use of P4 vaginal gel in pregnancy is safe. This indicates that the time of a universal recommendation to treat patients with a short cervix in midtrimester with vaginal P4 gel has not yet come. Additionally this demonstrates the importance of intense further research and also the need to investigate alternative ways of P4 supplementation concerning the choice of vehicle, the route of administration, the timing of treatment and the selection of the population.

Proposed mechanism of actions

There is a great body of evidence about the role and effects of P4 on cervical ripening that can be induced with antiprogestins in all species examined so far, including humans and primates (Chwalisz and Garfield, 1994). Supported by investigations of our group, there is growing evidence of direct actions of P4 on uterine contractility.

In the rat model we showed that P4 treatment delayed cervical ripening and completely blocked parturition (Figs. 1, 3, 6) (Kuon et al., 2010;2011). However, cervical ripening only was decelerated and not blocked and the cascade was finalized despite continual supplementation of P4. Assuming that both a ripe cervix and uterine contractions are required for successful delivery, it may be concluded that a block (i.e. inhibition) of uterine contractions is the responsible mechanism for delay of parturition in the rat model.

Furthermore treatment with onapristone, which is a pure antiprogestin, induces preterm delivery and increases the uterine electromyographic activity in rats (Fig. 2A) (Shi et al., 2008)). It has been suggested that P4 regulates parturition through genomic actions mediated by the nuclear P4 receptor that function as ligand-activated transcription factors and modulates various proteins that are thought to be involved in maintenance of pregnancy and initiation of labor (Garfield et al., 1980;1998). Moreover, recent studies indicate a direct suppression of uterine contractility by P4, but not 17P, by binding to membrane receptors through a non-genomic, rapid

mechanism and direct affection of cell function by modulating intracellular signal transduction pathways (Fig. 2B) (Ruddock et al., 2008). This inability of 17P to inhibit uterine activity has also been reported by others (Sexton et al., 2004). Recently different types of P4 receptors on the plasma membrane have been identified, and are thought to be responsible for these fast actions of P4 (Mesiano, 2004).

Our group investigated in in vitro studies the inhibition of uterine contractility with various tocolytics with and without P4 (Baumbach et al., 2012). P4 alone had little effect to inhibit contractility in uterine tissues from women undergoing cesarean section at term. However, P4 combined with the tocolytic drug nifedipine was significantly more effective than nifedipine alone. Further studies are essential to evaluate the potential use of P4 as a tocolytic therapy especially in combination with all the different tocolytic compounds that are on the market. However the first results of the studies are promising and encouraging for further research.

The myometrium is composed of billions of smooth muscle cells, which are coupled together electrically by gap junctions that are composed of connexin proteins, the most important of which is thought to be connexin 43 (Garfield et al., 1998). During the long period of pregnancy in which uterine quiescence is required, the density and concentration of these cell to cell contacts is low, indicating poor coupling and decreased electrical conductance. Finally at term this situation changes dramatically: the cell junctions increase, the cells become coupled and form a so called electrical syncytium that is thought to be essential for effective uterine contractions (Sims et al., 1982). The density of myometrial gap junctions is downregulated by P4, whereas P4 antagonists dramatically upregulate their expression (Garfield et al., 1998). However, as several hours of P4 administration is needed to observe these genomic effects, this mechanism can not account for the acute and fast acting tocolytic effects of P4 that have been described. As mentioned before no withdrawal of P4 hormone level can be detected in humans. Nevertheless changes of the P4 receptor in the decidua, amnion, and cervix have been shown and might account for a functional P4 withdrawal that corresponds to the fall of hormone levels in rodents (Zakar and Hertelendy, 2007).

One of the established pathways that lead to preterm cervical changes and contractions of the uterus are infections, which are associated with an increase of the release of proinflammatory cytokines such as interleukin (IL)-1beta, tumor necrosis factor-alpha and IL-8 that induce cervical ripening by activating the production of matrix metalloproteinases and thereby the degradation of the cervical collagen structure (Peltier, 2003). P4 has the ability to modulate antibody production and also to reduce the production of proinflammatory cytokines by direct and indirect interaction on immune cells and with this it might interrupt the cascade of inflammation and the irreversible degradation of the matrix that lead to softening and effacement (Kelly, 1994; Word et al., 2007).

S.c. administration of 17P also delayed cervical ripening (Fig. 4)., although less effectively than did P4 which indicates the importance of the choice of the type of progestin.

Route of administration

Recent reports that studied the effects of P4 supplementation focused on the vaginal route of administration (Romero et al., 2012). The vaginal route is thought to have some extra benefits when effects focused on the uterus are desired (De Ziegler et al., 1997). Not unlike the concept of the liver first-pass effect after administration of oral drugs, De Ziegler et al. established the term "uterine first-pass effect", that might be attributed to the network of arteries and veins linking the vagina to the uterus. This effect should favour an optimal uterine exposure after transvaginal treatment with sex steroids (Cicinelli et al., 2000). There are many studies of the uptake of P4 by the vaginal route that established the phenomenon of the uterine first-pass effect, but most of these studies were accomplished on postmenopausal women. Richardson et al. showed that the absorption of drugs across the vaginal epithelium is influenced by several physiologic factors, such as the properties of the epithelium and the volume, viscosity and pH of the vaginal fluid (Richardson, 1992). Thus the uptake of vaginally administered drugs may differ in pregnancy due to changes in the vagina epithelium or other factors. In our studies with P4, given by different routes and vehicles, we measured plasma P4 level changes at the end of pregnancy (Fig. 5).

Treatment with vaginal P4 did not inhibit the physiological P4 withdrawal, had no decelerating effect on cervical ripening and also failed to inhibit delivery in our study, which questions this route of administration, at least in rats (Figs. 3C, 3D, 5, 6). Vaginal R5020 blocked delivery effectively and possibly solved the problem of low uptake by the vaginal route by its high binding affinity to the P4 receptor (Fig. 6).

In this study oral P4 failed to inhibit delivery in rats probably because of a low uptake and the liver first-pass effect (Fig. 6). The main advantage of oral administration of P4 is its non-invasiveness and consequent acceptability. However, there is a high

variation of absorption of oral P4, and the metabolism by the first-pass effect in the liver is rapid, which makes the oral administration essentially ineffective. Moreover, considerable side effects such as intrahepatic cholestasis, sleepiness, fatigue, headaches and nausea are more common when P4 is given orally (O'Brien et al., 2007; Rode et al., 2009). Even sedative and hypnotic effects or fluid retention have been described. Three randomized trials compared oral P4 to placebo for prevention of preterm birth. In the studies published in 1986 and 1991, oral P4 did not prolong gestation in patients treated for preterm labor (Erny et al., 1986; Noblot et al., 1991). In contrast, Rai et al. reported a reduction in preterm delivery and also a reduction in neonatal morbidity and mortality in women with a history of at least one preterm birth who received oral P4 from recruitment (18-24 weeks) until 36 weeks or delivery (Rai et al., 2009).

Transdermal supplementation of P4 to prevent preterm birth has not been studied in humans although it is quite common to use this route for the application of steroids in humans for other indications. Sitruk-Ware explained that this route may additionally help administering other progestins that are inactive when given orally (Sitruk-Ware, 2007). For example the progestin nestorone (a 19-norprogesterone derivative), that possess a high specificity for binding to the P4 receptor with no or little interaction with other steroid receptors, is rapidly metabolized and therefore inactive after oral administration but shows a high bioavailability after transdermal application and thus promising studies are underway to investigate transdermal gels and sprays for contraception (Sitruk-Ware and Nath, 2010). R5020, the 19-norprogesterone that we used in our animal study, blocked parturition very effectively, also in very low doses and might additionally be a candidate for transdermal application. Furthermore, depending on the carrier, the transdermal route was associated with a high uptake and plasma P4 levels and was very effective in postponing delivery in rats (Figs. 5, 6).

Holzer et al. (2005) investigated the effects and side-effects of P4 cream on the female skin. They described minimum side-effects, which also occurred in the placebo group and therefore they concluded that the vehicle used might be responsible for the skin irritations. Additionally the study reported that topical P4 improved the visco-elastic properties and firmness of the skin significantly, which might also help to reduce the appearance of stretch marks as a positive side-effect. Thus this route of administration should be considered as a non-invasive, easy to administer alternative and might become the focus of future research.

Because of the ineffectiveness of oral P4, luteal support in infertility treatments was initially given by intramuscular injections but is now abandoned due its side-effects, such as patient discomfort, inflammatory reactions and even abscesses (Kleinstein, 2005). The effectiveness of intramuscular injections of P4 to prevent preterm birth has not been evaluated in clinical trials. The reason for this is that daily intramuscular injections would be required to maintain therapeutic serum levels due to the relatively short half-life of P4. This would make this intervention very invasive, especially if P4 was to be given by prolonged prophylactic administration to women at increased risk for preterm birth.

17P is a long-acting progestin and is given exclusively intramuscularly in clinical trials Even with weekly intramuscular injections, however, side-effects such as injection-side pain, swelling, itching and bruising have been reported in up to one-third of treated women, and were more common in the progestin group as compared to placebo (Meis et al., 2003). Our data supports the view that 17P is a weak progestin and thus unlikely to be useful for preterm labor treatment.

More data is needed before any formulation and route of P4 administration for prevention of preterm birth can be recommended over the others. In our study only s.c. injections of P4 and transdermal administration of P4 in sesame oil (partly) and in fish oil (completely) (not in Replens®, see below) blocked delivery (Fig. 6). Notably, none of these routes has been used in clinical trials to date. On the other hand, oral P4 and vaginal P4 administration, studied in humans so far, have not had any effect on time of delivery in our study in rats. Concluding from recent studies that showed some minor effects on preterm birth after vaginal treatment with P4 it is possible that the effects of topical or s.c. P4 might be far superior.

The importance of the vehicle

As described above the favoured pathway of administrating P4 in the recent studies has been the vaginal route. Hassan et al. used a gel (Crinone®) utilizing a bioadhesive, biocompatible polymer as a base (Replens) that is believed to attach to the vaginal epithelium with proposed advantages such as facilitated diffusion of P4 across the vaginal epithelium to accomplish a sustained release effect. Another way to administer P4 vaginally is the use of capsules/tablets, which originally have been designed for oral administration. These capsules are difficult to administer and are associated with discomfort (itching, burning), local irritation of the vaginal mucosa (e.g. erythema) and vaginal discharge, adverse effects which are also seen after treatment with Crinone® (Kleinstein, 2005). Kleinstein et al.

compared the efficacy and tolerability of vaginal P4 capsules (Utrogest® 200, a soft gelatin capsule containing P4 suspended in oil) with P4 gel (Crinone® 8%). Both treatments resulted in similar outcomes with respect to implantation, ongoing pregnancy, and abortion rates. In another study, Kleinstein et al. compared the bioavailability of Utrogest® 200 to that of Crinone® in healthy young women (Kleinstein et al., 2002). The duration of peak levels and the bioavailability was higher with Utrogest® 200 which question the superiority of the vaginal gel that is often assumed. The adverse effects of the mentioned vaginal treatments might be resolved by a vaginal ring that releases P4 continuously. Vaginal rings that deliver hormones were developed for contraception and hormone therapy and have been very successful by combining many advantages that might also be important in the context of a treatment for preterm birth: high acceptability and user satisfaction as the long-acting system can be easily administered by the patient, it needs no daily attention or administration as it stays in place without being noticed. Additionally the problem of a discontinuous supply of the drug due to a system that needs replacement might be resolved with a vaginal P4 ring.

There are numerous topical P4 fabricates with different characteristics on the market to treat symptoms in postmenopausal women. The uptake of P4 is greatly influenced by the characteristics of the formulation including P4 concentration and solubility of the formulation, physical and chemical properties of ingredients, the effect on the integrity of the skin and also the site and surface area of the application (Stanczyk et al., 2005).

In our study, topical micronized P4 in oil but not in Replens®, the vehicle of the vaginal P4 gel Crinone®, was very effective to inhibit delivery and also to forestall the withdrawal of P4 at the end of gestation (Figs. 5, 6). Again this indicates the importance of the vehicle, also for the transdermal route of administration.

Conclusion

There is growing evidence for beneficial effects of P4 as a treatment of preterm birth. However present clinical studies are still controversial and pose serious questions about the effectiveness of Crinone®. Our own studies demonstrated:

1. The vaginal route is not the best route of administration. Topical (transdermal) administration is better. Topical micronized P4 in fish oil is an effective route to block delivery and might be a novel route of administration in clinical studies to treat preterm birth.
2. The base used for the vaginal gel preparation (Replens®) is not appropriate because when it is used topically it does not allow uptake and inhibit delivery.
3. The choice of the progestin is crucial. P4 and R5020, but not 17P, inhibit delivery in rats. More effective treatment of preterm birth might be achieved with a more potent progestin (as we show for R5020).
4. P4 is able to block uterine contractions. Inhibition of birth is primarily due to inhibition of uterine contractility and not to a block of cervical ripening.

The route of administration and the vehicle is critical and ongoing research should not limit the investigations to the vaginal route of administration, as the alternatives, in particular the transdermal application of P4, might be promising. Further studies are indispensable to address the effects of a P4 supplementation given by other routes and in other vehicles. Additionally, as P4 successfully suppresses uterine contractions in our experiments, its application as a tocolytic agent to treat the acute symptoms of preterm labor needs to be studied intensely.

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