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5β-Dihydroprogesterone and Human Preterm Labor

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Abstract

We previously investigated the progesterone metabolite 5β-dihydroprogesterone (5βDHP) in relation to human parturition at term, demonstrating that peripheral venous concentrations decrease in association with the onset of spontaneous labour. In this study our aim was to determine if 5βDHP concentrations were lower in women presenting in spontaneous preterm labour than in controls matched for gestational age. Blood samples were obtained from women presenting in spontaneous preterm labour (n = 20). The diagnosis was made on the presence of regular contractions and cervical effacement and dilatation of at least 3 cms. All women in the preterm labour group delivered before 37 weeks gestation. Blood samples were then obtained from controls, closely matched for gestational age with uncomplicated pregnancies. The preterm labour group was further stratified by cause into three groups, chorioamnionitis (n = 5), abruption (n = 4) and idiopathic (n = 11). Following organic solvent extraction, steroids were separated by HPLC and 5βDHP quantified by radioimmunoassay. Women in the idiopathic preterm labour group were found to have significantly lower circulating concentrations of 5βDHP than controls ($p < 0.05$). There were no significant differences found in either the chorioamnionitis or abruption groups. This result suggests that there may be a group of women in whom the normal endocrine process of labour is triggered early resulting in preterm labour.

Keywords

5β-Dihydroprogesterone, Human Parturition, Progesterone Metabolites, Preterm Labor

1. Introduction

Spontaneous preterm birth accounts for up to 15% of the perinatal mortality in infants born without congenital

abnormalities. Possible consequences of severe prematurity include cerebral palsy, developmental delay, visual and hearing impairment and chronic lung disease. Despite efforts to detect and prevent preterm labour and delivery, rates for singleton deliveries have remained static in developed countries. In Victoria, Australia, the commonest cause of neonatal death in the absence of congenital abnormalities (birthweight of 400 g or greater) was spontaneous preterm delivery, accounting for 32.6% of neonatal deaths [1].

Preterm labour is known to be a heterogeneous condition with a number of different causes. Recent studies have identified four common pathways leading to preterm labour and delivery; inflammation, decidualhaemorrhage, uterine over-distension and premature activation of the normal physiologic initiators of labour [2]. A number of predictive tests are currently used in an attempt to identify those women who will deliver preterm, including studies of uterine contractions, fibronectin, and ultrasound assessment of the cervix. The effectiveness of these tests is limited by our lack of knowledge of the normal processes of parturition.

While the importance of progesterone in the maintenance of uterine quiescence during most of pregnancy has been demonstrated in many species, no mechanism for progesterone withdrawal leading to labour onset in late pregnancy has been clearly defined in humans. It is uncertain if progesterone has an acute tocolytic effect when used to treat patients with preterm labour, though prophylactic administration may decrease the incidence of preterm labour in high-risk women [3] [4]. Grazzini *et al*. proposed that a progesterone metabolite, 5β-dihydroprogesterone (5βDHP) may be responsible for maintenance of uterine quiescence throughout pregnancy [5]. Although the finding that 5βDHP binds to the human oxytocin receptor has not been confirmed by other investigators, [6] [7] the ability of 5βDHP to prevent spontaneous myometrial activity *in vitro* has been described [8] [9]. We have previously demonstrated a decrease in circulating 5βDHP concentrations in women during spontaneous labour at term and also a significant decrease in 5β-reductase mRNA in myometrium and placenta in association with human term spontaneous labour [10] suggesting a possible role for this metabolite in the onset of labour at term. We therefore investigated plasma concentrations of 5βDHP in women presenting to hospital in preterm labour.

The aim of this study was to compare plasma 5βDHP concentrations in women presenting in preterm labour with women at matched gestations not in labour. Our hypothesis was that 5βDHP concentrations would be lower in women experiencing preterm labour.

2. Subjects and Methods

2.1. Subjects

Blood samples were obtained from twenty patients between 24 weeks' gestation and 36 weeks' gestation who presented with preterm labour. The diagnosis was made on the basis of a history of painful contractions and cervical dilatation of at least 2 cm and full effacement. None of the patients had any past history of preterm labour or obvious risk factors for preterm labour. None of the patients had preterm prelabour rupture of the membranes (PPROM) at the time of presentation. The blood samples were all taken within 5 hours of admission to hospital. All of these patients delivered before 37 weeks gestation although the time interval from the taking of the blood sample to the time of delivery varied from 30 minutes to 14 days. All of the patients received at least one dose of intramuscular betamethasone for fetal lung maturation prior to delivery. All of the patients received some form of tocolytic, either intravenous salbutomol or oral nifedipine.

Preterm labour patients were separated into three groups on the basis of clinical presentation, biochemistry and placenta histology. Patients in the abruption group presented with an obvious clinical abruption and had evidence of placental ischaemia and abruption on placental histology. Patients in the chorioamnionitis group were either febrile > 38˚C on admission, had a markedly raised C-reactive protein (over 100 mg/L, normal values 0 - 9 in pregnancy) or evidence of chorioamnionitis on placental histology. Patients included in the idiopathic group had none of the above findings.

A blood sample was then obtained from a patient with an uncomplicated pregnancy, not in labour at a gestation as closely matched as possible to that of the patient with preterm labour. Gestations were matched within a maximum of five days. All of these control patients delivered after 38 weeks completed gestation. There were no significant differences in maternal age or parity between the two groups. Three patients in the idiopathic preterm labour group had a history of a prior preterm labour and delivery as did one patient in the ischaemic group. One of the latter group controls also had a history of preterm labour.

All subjects gave written informed consent to participate in the study, which was approved by the Research

and Ethics Committees of the Royal Women's Hospital, Melbourne, where the patients were delivered.

Information regarding patient demography is summarized in **Table 1** and **Table 2**. **Table 1** relates only to the cases while **Table 2** includes data pertaining to both cases and controls. Patients in the idiopathic preterm labour group were at significantly later gestation compared with the other two groups.

2.2. Patient Sampling

Blood was collected by venepuncture into 9 ml EDTA tubes and stored at 4˚C until centrifuged at 1800 g for 10 minutes within 3 hours of collection. Plasma was collected and stored as 1 ml aliquots at −40˚C until assayed.

2.3. Determination of 5βDHP

The method used has been described in detail in a previous publication [10]. Plasma aliquots of 1 ml were extracted with hexane (HiPerSolv for HPLC, BDH, Quebec, Canada). The organic phase was removed using a glass pasteur pipette, evaporated to dryness under nitrogen and resuspended in acetonitrile (HiPerSolv for HPLC, BDH, Quebec, Canada).

Using a Shimadzu LC10A High Performance Liquid Chromatograph, including two pumps and an autoinjector module, 100 µl of extract was eluted isocratically with acetonitrile and water (60:40; 1 ml/minute) for 30 minutes at room temperature according to the method described by Walters *et al*. [11]. Fractions of 1 ml were collected in Eppendorf tubes by a Shimadzu FRC-10A fraction collector each minute between 10 minutes and 20 minutes. Standard solutions of various steroids (including progesterone and 5βDHP), dissolved 1 mg/ml in acetonitrile were run after the samples. Steroids were obtained from Sigma (MO, USA). The fraction containing 5βDHP and three surrounding fractions were dried under nitrogen and resuspended in phosphate buffer. A 100 µl aliquot of each fraction was assayed by radioimmunoassay (RIA) using a cross-reacting progesterone antibody obtained from Bioquest, (NSW, Australia). Precipitate was resuspended in 2 ml of scintillant (Starscint,

Table 1. Demographic data.

Group	Maternal age mean	Parity median	Gestation (no. of weeks) mean* (range)
Idiopathic—case	30	0	33 (30 - 34)
Idiopathic—control	29	0	33 (30 - 35)
Chorioamnionitis—case	31	0	28 (23 - 34)
Chorioamnionitis—control	31	0	28 (23 - 34)
Ischaemic—case	29	0	31 (28 - 32)
Ischaemic—control	27	0	31 (28 - 32)

*Significant difference between idiopathic/chorioamnionitis/abruption groups $p < 0.001$ by ANOVA

Table 2. Demographic data for cases and controls. There were no statistically significant differences between the groups for maternal age, parity or time from sample collection to delivery.

	Maternal Age (mean)	Parity (median)	History of preterm labour (no. of patients)	Time from sample taking to delivery in hours (mean, range)
Idiopathic—case	30	0	3	22, 0.5 - 192
Idiopathic—control	29	0	0	N/A
Chorioamnionitis—case	31	0	0	61, 1 - 336
Chorioamnionitis—control	31	0	0	N/A
Ischaemic—case	29	0	1	81, 7 - 192
Ischaemic—control	27	0	1	N/A

Packard Bioscience, CTUSA) and radioactivity was quantified by scintillation spectrometry in a Wallac 1409 liquid scintillation counter. The standard curve was calculated and concentrations of individual samples estimated by the MultiCalc on-line software. The cross-reactivity for key steroids progesterone, 5α-dihydroprogesterone (5αDHP) and 5βDHP were checked under the described laboratory conditions and found to be 100%, 62.5% and 25% respectively.

Intra-assay CV was determined to be 5% +/− 0.1% on 5 samples. (All results are given as mean +/− SEM). Inter-assay CV was estimated at 14% +/− 0.8% on 10 assays. The effects of inter-assay variation were minimized by ensuring that samples for comparison were measured in the same assay.

2.4. Recovery

Recovery of steroids from the plasma sample was estimated by addition of 72 kBq/tube of tritiated progesterone tracer in 10 µl of phosphate buffer to the original plasma sample, which was then extracted, resuspended and eluted through the HPLC column as described above. The second fraction, corresponding to the progesterone standard peak was then dried and re-suspended in buffer as described and a sample of this quantified by scintillation spectrometry. Using this method, mean recovery was estimated as 71.5% +/− 2% (n = 2).

2.5. Statistical Analysis

Patients' results were compared with controls matched for gestational age using a 2 tailed t test presupposing unequal variance. Correlation of 5βDHP concentrations and time to delivery was by calculation of the correlation coefficient. Correlation of 5βDHP concentrations and gestational age was by means of nonparametric testing. Demographic data for the three groups was compared using analysis of variance (ANOVA).

3. Results

The preterm labour patients were clearly divisible into three groups according to aetiology as suggested by clinical findings and biochemical and histological investigations. When compared with non-labouring controls matched for gestational age, patients in idiopathic preterm labour had significantly lower plasma 5βDHP concentrations (**Table 3**, **Figure 1**). There was no correlation between 5βDHP concentrations and the timing of the sample before delivery.

Patients presenting with chorioamnionitis showed no difference in 5βDHP concentrations (**Figure 2**). Analysis of the abruption group also demonstrated no significant difference between patients and matched controls (**Figure 3**).

The effect of increasing gestation on circulating 5βDHP concentration was considered. Cases were excluded from this analysis as it is possible that the pathological conditions represented may influence the results. There was no significant correlation found between 5βDHP concentration and gestational age (**Figure 4**).

4. Discussion

On the basis of our current understanding of the onset of human parturition, it seems probable that there are a number of different pathways leading to the onset of labour depending on circumstances such as infection. The division of the patients in this study into three different groups is in accord with current theories of the heterogeneous nature of the condition of preterm labour.

The finding of a significantly later mean gestation at delivery in the idiopathic preterm labour group is also in accord with previous studies of markers of preterm delivery. In other studies of preterm labour, inflammation is more often associated with preterm births before 32 weeks, whereas decidualhaemorrhage may occur at any time. Premature onset of apparently normal labour is typical of preterm labour after 32 - 34 weeks [2]. Controls were all closely matched for gestation so any difference between groups is unlikely to have an impact on the results obtained. Idiopathic preterm labour was obviously the most common cause. As a result, the chorioamnionitis and ischaemic groups contain very small numbers, in particular the latter which may impact on the ability of the study to find significant differences in these groups.

In addition to matching for gestation, the cases and controls were also closely matched for other demographic data such as maternal age and parity. The mean time from obtaining a blood sample to delivery was shorter in the idiopathic group but there was no statistically significant difference between the three groups by ANOVA.

Table 3. Results.

	Labour status	Plasma 5β-DHP (mean +/− SEM nmol/ml)	Mean difference and significance (nmol/ml)
Idiopathic preterm	Preterm labour	0.186 +/− 0.022	
(n = 11)	Not in labour	0.275 +/− 0.037	0.088 ($p < 0.05$)
Chorioamnionitis	Preterm labour	0.211 +/− 0.065	
(n = 5)	Not in labour	0.208 +/− 0.052	0.003 Not significant
Abruption	Preterm labour	0.118 +/− 0.034	
(n = 4)	Not in labour	0.130 +/− 0.050	0.012 Not significant

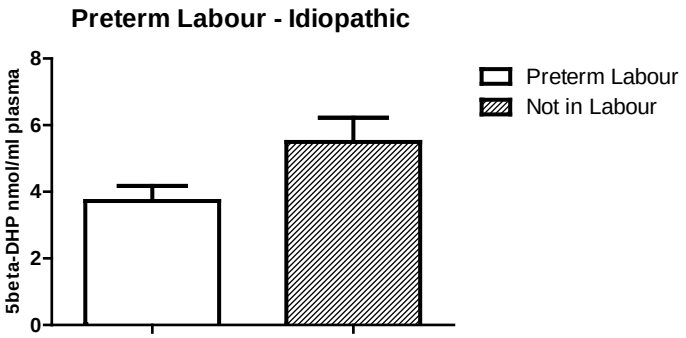

Figure 1. Idiopathic preterm labour (n = 11). Error bars show standard error.

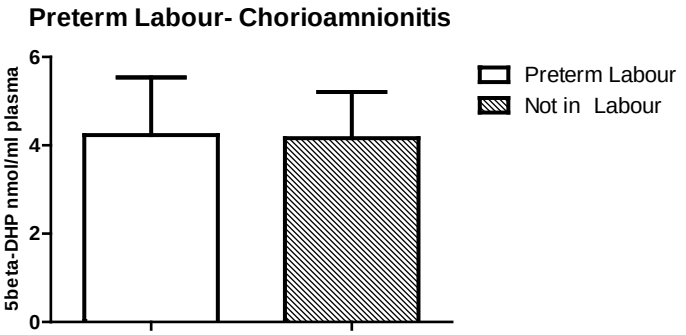

Figure 2. Preterm labour due to chorioamnionitis (n = 5). Error bars show standard error.

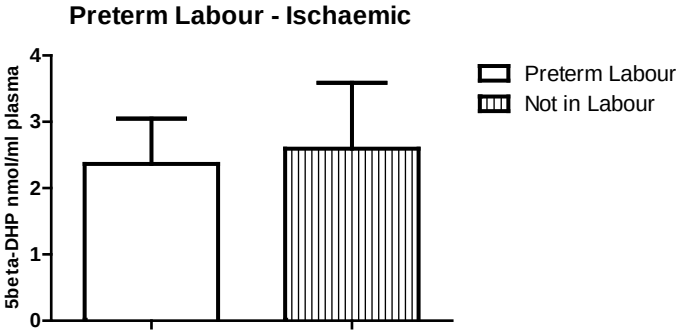

Figure 3. Preterm labour due to ischaemia/placental abruption (n = 4). Error bars show standard error.

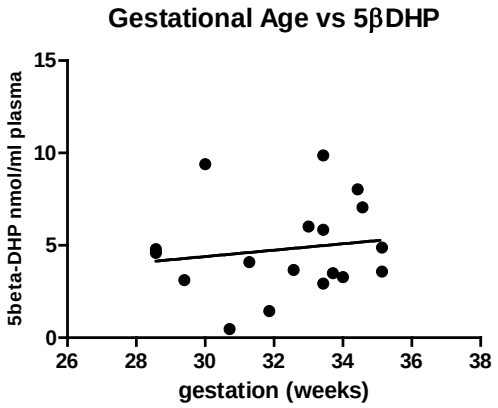

Figure 4. The effect of gestational age on 5*β*DHP concentration. Cases have been excluded from the analysis (p = 0.48).

There was no correlation with the absolute concentration obtained and the time to delivery for any of the samples. This is consistent with the high degree of individual variation in circulating steroid hormone concentrations which has been documented for other steroid hormones including progesterone and estradiol.

A detailed description of changes in circulating 5*β*DHP concentrations throughout pregnancy has not been published. One investigator has reported concentrations of progesterone metabolites from three different points in pregnancy and demonstrated that circulating concentrations of 5*β*DHP rise 5-fold by 37 weeks gestation compared early pregnancy concentrations however most of this rise occurs over the first and second trimesters with an apparent plateau from about 20 weeks gestation [12]. More detailed studies of related progesterone metabolites, the pregnanolone isomers, have demonstrated that 5*β*-reduced metabolites show a marked increase in concentration from the first month of pregnancy [13]. Concentrations reach a plateau around six months of gestation. These findings, using the technique of GC-MS, are directly comparable to the findings of the current study (**Figure 4**). It was apparent from this study that 5*β*-reduced metabolites did not simply follow the increasing concentrations of progesterone, suggesting that 5*β*-reduction is independently regulated during pregnancy. Our previous studies have demonstrated a significant change in both circulating 5*β*DHP concentrations and 5*β*-reductase mRNA in myometrium and placenta in association with term parturition [10] and 5*β*-reductase activity has been identified in myometrium [14] [15]. Although the liver is known to be the main site of 5*β*-reductase activity, metabolites produced by this pathway are thought to undergo immediate further metabolism and excretion [16] [17]. Circulating 5*β*DHP is thus likely to originate in extrahepatic sites of progesterone metabolism such as reproductive tract tissues which may be in keeping with the finding of differences in circulating concentrations with onset of labor. We found no correlation between absolute 5*β*DHP plasma concentrations and the subsequent time to delivery. This is in accord with both our findings in the previous study of 5*β*DHP concentrations in association with spontaneous labour at term in which considerable individual variation was noted and also with known data about individual variation in other plasma steroid hormone concentrations in pregnancy.

This is the first reported study of 5*β*DHP concentrations in association with preterm labour. We previously described a decrease in plasma concentrations of 5*β*DHP with the onset of the active phase of labour in normal women at term. As with the prior finding of a decrease in plasma concentrations in association with the spontaneous onset of labour of another progesterone metabolite [18], 5*α*DHP, this may be a typical endocrine change occurring as part of the normal human parturition process. In the current study, we identified a group of women in preterm labour who have lower concentrations of the progesterone metabolite 5*β*DHP than women at matched gestations not in labour. Furthermore, the onset of preterm labour in this group of patients was unexplained by other commonly described causes such as infection or abruption. From this, it is possible to conclude that there is a group of patients in whom the normal endocrine processes leading to parturition are triggered early. This may be linked to a genetic risk factor, such as an enzyme defect. A population based study has found that women with a prior preterm singleton delivery have a significantly increased risk of recurrence [19]. The development of earlier and more reliable markers of the onset of parturition may assist in the identification and man-

agement of this group of patients.

It is not clear whether the decrease in plasma concentration of the progesterone metabolite, 5βDHP, is a consequence of the endocrine process leading to the onset of labour in the human or a causal factor. This question must await the outcome of further research.

The mechanism of human parturition has proved challenging to describe. Research to date suggests that possible components include hormonal mediators estradiol, progesterone and its metabolites, estradiol and progesterone receptor conformation and expression and possible endogenous antagonists, regulation of placental CRH and inflammatory mediators, cytokines and prostaglandins. In such a complex system it is likely to contain multiple entry points and redundancies. Our results suggest just such a finding; that the presence of infection or blood products is able to trigger parturition by a different pathway to the usual mechanism at term.

Current tests used to predict preterm labour include cervical length on transvaginal ultrasound scanning, testing for fetal fibronectin in cervicovaginal secretions and maternal salivary estriol. These tests have different sensitivities and specificities depending on whether they are used in the presence or absence of symptoms of preterm labour. All have limitations in their use in current clinical practice. As our understanding of parturition improves it is to be hoped that more tests for preterm labour in different clinical settings will become available.

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