Fetal Plasma Prostaglandin F$_{2\alpha}$ and Cortisol Responses to High-Dose Endotoxin Administration in Fetal Goats

Atsushi Miura, Yoshio Yoneyama, Rintaro Sawa and Tsutomu Araki

Department of Obstetrics and Gynecology, Nippon Medical School

Abstract

Intrauterine inflammation/infection has been associated with prenatal mortality and morbidity. However, few studies have been performed to investigate how the fetus responds to intrauterine inflammation/infection in utero. In the present study, fetal plasma prostaglandin (PG) F$_{2\alpha}$ and cortisol responses to high-dose fetal endotoxin administration were evaluated in late gestation goats ($n=8$). After 160 $\mu$g/kg of fetal weight of endotoxin (Escherichia coli, O 111: B 4 lipopolysaccharide) administration via the fetal jugular vein over a 5-min period, fetal plasma PGF$_{2\alpha}$ and cortisol levels, fetal blood gases and pH were measured periodically. After endotoxin administration, fetal plasma cortisol levels significantly increased to 9.5 ± 0.8 ng/mL and 9.3 ± 0.7 ng/mL after 1 and 3 h, respectively ($p<0.05$) and plasma PGF$_{2\alpha}$ levels did not change throughout the study. These results suggest that absent PGF$_{2\alpha}$ and attenuated cortisol responses to high-dose fetal endotoxin administration, relative to the adult, may be a self-protective mechanism that diminishes premature delivery and fetal asphyxia.

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Key words: fetus, endotoxin, prostaglandin F$_{2\alpha}$, cortisol

Introduction

Prenatal exposure to inflammation/infection has been associated with numerous adverse outcomes that include abortion, premature delivery and increased risks of neonatal asphyxia and cerebral palsy$^{12}$. It has now been established that endotoxins stimulate production of cytokines in many infections$^1$. These in turn result in the secretion of prostaglandin (PG) F$_{2\alpha}$, which is responsible for uterine contraction$^1$ and cortisol, which is considered to determine the onset of labor pain in the fetal period$^1$.

The hypothalamo-hypophyseal-adrenal axis is almost established at 100 days gestation in fetal goats, which is slightly different from the time in sheep and cows. However, all of them accord with late gestation in human.

In previous studies, maternal endotoxin administration elevated fetal plasma PGF$_{2\alpha}$ and cortisol, which are, at least in part, associated with abortion, premature labor, fetal asphyxia or death$^{1-3}$. However, the effects of fetal exposure to endotoxin in pregnancy outcome has not been clearly elucidated. Fetal plasma PGF$_{2\alpha}$ and cortisol responses to infusion of medium doses of endotoxin into goat fetuses (80 $\mu$g/kg of estimated fetal weight) have been examined only in our previous study$^3$. We found that absent fetal PGF$_{2\alpha}$ and
attenuated cortisol responses to fetal endotoxin administration, relative to the adult. However, the effects of high-dose endotoxin administration into the fetus on fetal plasma PGF_2α and cortisol levels have not been evaluated in late gestation goats.

The present study was designed to evaluate the extent of the effects of administration of high-dose endotoxin (160 μg/kg of estimated fetal weight) to the fetus on fetal plasma PGF_2α and cortisol release in late gestation goats.

Materials and Methods

1. Surgical procedures

Eight pregnant mixed-breed goats mated on only a single occasion and of known gestational age (126 ~ 135 days; Term=150 days) were used in the study. The animals were housed in rooms with controlled light cycles. They were fed each morning with alfalfa cubes and had free access to water at all times.

Surgery was performed on 8 pregnant mixed-breed goats. After a 24-h fast, the goats were anesthetized with a mixture of 1.5~25% halothane and oxygen. Under aseptic conditions the maternal skin and uterus were incised and the fetal head was delivered. Polyvinyl catheters (1.0 mm ID) were inserted into the fetal carotid artery and jugular vein and their tips advanced into the aortic arch and superior vena cava, respectively. An additional catheter was placed in the amniotic cavity for administration of antibiotics. Then, the fetal body was replaced into the uterus. All catheters were exteriorized through an incision in the maternal flank and protected in a nylon pouch. The goats were kept in metabolic carts and were allowed to recover for at least 4 days before experiments were begun.

Ampicillin was administered to the maternal goats on a daily basis (4 g/day, iv), and given to the fetus via instillation into the amniotic cavity (1 g/day).

2. Experimental protocol

This study was approved by the Ethical Committee of the Hobara Central Hospital, Fukushima, Japan. Experiments were started at 4 days after surgery. Prior to the start of each experiment, samples for measurement of fetal arterial pO₂, pCO₂ and pH were taken. Only fetuses with arterial blood gas and pH values in the normal ranges for our laboratory (pO₂>17 mmHg and pH >7.28) were studied.

The protocol consisted of control and after endotoxin administration periods. After a 1-h control period, *Escherichia coli* endotoxin (160 μg/kg of estimated fetal weight) (O111:B4 lipopolysaccharide, Wako, Tokyo) was dissolved in 3 mL of sterile saline and infused into the fetal jugular vein over a 5-min period. Eight control fetal goats received an infusion of saline (3 mL) via the fetal jugular vein.

After endotoxin or saline administration, the condition of the fetal goats was monitored for the next 12 hours. Fetal arterial blood pressure was measured with a pressure transducer (Cobe laboratories, Lakewood, CO) and heart rate was determined with a cardiotachometer triggered by the arterial pulse pressure. Fetal arterial pressure and heart rate were recorded to a stripchart recorder (RM-6366, Nihon Koden, Tokyo, Japan), and values were sampled every 0.01 sec with an NEC computer, with minute averages stored on disk. Fetal plasma PGF_2α and cortisol levels, fetal blood gases and pH were measured periodically.

3. Blood sampling

Fetal blood samples were collected from the carotid artery at 30 min before the endotoxin or saline administration, and 0.5, 1, 3, 5, 9 and 12 h after endotoxin or saline administration. The first portion of each fetal blood sample (0.4 mL) was taken into an ice-cold heparinized syringe. Fetal blood gases and pH were measured (Model 148, CIBA-Corning, Medford, MA).

The next sample (2 mL) was taken into an ice-cold heparinized syringe, immediately transferred into an ice-cold centrifuge tube, and mixed with 100 μL of an aqueous solution containing EDTA (3 mM) and indomethacin (0.2 μg/mL) to prevent in vitro formation of prostaglandins. Samples were centrifuged at once (1,300 g for 5 min, 4°C). The plasma was collected and stored at −80°C until analysis. Plasma PGF_2α level was determined by
a specific radioimmunoassay\textsuperscript{11}. The detection limit of the assay was 2.5 pg/mL. There was no cross-reactivity between PGE\textsubscript{2} and PGF\textsubscript{2α}. The intra- and interassay coefficients of variation of PGF\textsubscript{2α} assays were less than 5.2\% and 6.8\%, respectively.

Plasma cortisol level was measured by radioimmunoassay after methylene dichloride extraction, as described previously\textsuperscript{1}. The intra- and interassay coefficients of variation were 6.3\% and 7.4\%, respectively. The assay sensitivity was 3.0 ng/mL.

The dose of endotoxin in this study (160 μg/kg of estimated fetal weight) was determined by a preliminary study (n = 10). A higher dose of endotoxin (200 μg or more/kg of estimated fetal weight) killed the fetuses suddenly without affecting fetal cardiovascular conditions in the preliminary study.

4. Statistical analysis

Data are presented as mean ± standard error of the mean (SEM). Student’s t test was used to determine significant differences for single comparisons. Analysis of variance was used to determine the significant difference for repeated measures. If overall significance was observed, then individual group means were compared by the Bonferroni’s post hoc multiple comparison test. Differences were considered significant at p<0.05.

Results

1. Changes in fetal plasma PGF\textsubscript{2α} levels

Changes in fetal plasma PGF\textsubscript{2α} levels are shown in Fig. 1. Mean plasma PGF\textsubscript{2α} levels after endotoxin and saline administration averaged 604±61 pg/mL and 581±63 pg/mL, respectively, during the control period, and did not change during the remainder part of study. Plasma PGF\textsubscript{2α} levels in endotoxin-administered fetuses were not significantly different from those in control fetuses throughout the study.

2. Changes in fetal plasma cortisol levels

Changes in fetal plasma cortisol levels are shown in Fig. 2. In endotoxin-administered fetuses, plasma cortisol averaged 8.1±0.6 ng/mL during the control period, and then significantly increased to 9.5±0.8 ng/mL and 9.3±0.7 ng/mL after 1 and 3 h, respectively (p<0.05). During the remainder of the study, fetal plasma cortisol returned to basal levels. Plasma cortisol levels in endotoxin-administered...
fetuses at 1 and 3 h were significantly different from those in control fetuses (p<0.05). Plasma cortisol levels in control fetuses did not change throughout the study.

3. Fetal blood gases and pH

Table 1 shows the changes in fetal heart rate, mean arterial blood pressure, blood gases and pH. In endotoxin-administered fetuses, fetal blood pO₂ and pH significantly decreased after 3 and 5 h, and 5 h (p<0.05), respectively and pCO₂ significantly increased after 3 h (p<0.05). These values returned to initial levels after 12 h. However, the mean values of these variables remained within normal physiologic ranges. Fetal heart rate or mean arterial blood pressure did not change throughout the study. Control fetuses did not show changes in measured variables after saline administration.

Discussion

In this study we have shown that fetal absent PGF₉ and attenuated cortisol responses to high-dose endotoxin administration to the fetus in late gestation goats. To date, the effects of high dose endotoxin on fetal PGF₉ₐ and cortisol release have not been evaluated in late gestation goats.

In the present study, high-dose endotoxin administration to the fetus did not alter fetal plasma PGF₉ₐ levels. To date, fetal goat plasma PGF₉ₐ response to fetal endotoxin administration has been reported only in our previous study². In that study, we infused medium doses of endotoxin to the fetuses (80 μg/kg of estimated fetal weight), which also demonstrated absent PGF₉ₐ response in fetal goats. Since we administered two times higher dose of endotoxin in the present study, which is in the region of a lethal dose², the results observed here indeed reflect absent fetal PGF₉ₐ response to endotoxin.

The exact mechanism of absent fetal PGF₉ₐ response to endotoxin has not been elucidated. One possible explanation is that fetal plasma PGF₉ₐ level is maximally elevated in late gestation², so it is no longer able to respond to endotoxin stimulation.

Further peripheral levels of PGF₉ₐ do not necessarily reflect those in the action site, such as hypothalamo-hypophyseal axis. Further study is needed to clarify the mechanism of the absence of a response to endotoxin in the fetus.
Table 1  Changes in fetal heart rate, mean arterial blood pressure, blood gases and pH after endotoxin or saline administration

<table>
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<th>9h</th>
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<td>164 ± 17</td>
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<td>172 ± 12</td>
<td>170 ± 24</td>
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Date are presented as mean ± SEM. MABP, mean arterial blood pressure.
*p < 0.05 significant from the control period (-0.5h) and control fetuses.

In this study, fetal plasma cortisol levels slightly elevated after endotoxin administration. Fetal plasma cortisol response to maternal endotoxin administration has been reported in a few studies\(^{18}\), which indicated that the extent of elevation of fetal plasma cortisol levels in previous and the present study was two to ten-fold less than that measured in their mothers.

The plasma cortisol response of fetal goats to high-dose endotoxin administration to the fetus has not been investigated to date. An attenuated cortisol response to medium doses of endotoxin administration to the fetus was observed in our previous study\(^{19}\), and the same findings were observed after high-dose endotoxin administration in the present study, so the attenuated cortisol response observed in this study indeed reflects attenuated fetal cortisol response to endotoxin. The mechanisms of attenuated fetal plasma cortisol response are unknown. One possible explanation is that the fetus is generally less sensitive to endotoxin than is the adult\(^{20}\). Further study is needed.

In this study, the fetal cardiovascular system did not respond to endotoxin, and fetal blood gases after endotoxin remained within the normal range. These results are in accord with the previous study on pregnant sheep and goats\(^{14,15}\). These absent or attenuated responses in previous and the present study may be related to the immature adrenergic system, vascular shunt and poor responsiveness to endotoxin in the fetuses.

Maternal endotoxin administration increases fetal plasma PGF\(_2\alpha\)\(^{16}\) and cortisol\(^{17}\) levels which, at least partly, may be involved in the induction of abortion and onset of premature delivery. Further, endotoxin itself may be associated with fetal endotoxin shock under some conditions\(^{18}\). Therefore, the absent or attenuated sensitivity of the fetus to fetal endotoxin observed in this study may be beneficial for fetuses to maintain stable conditions in utero. However, it has been evident that infection in the pregnant uterine cavity may be chronic and remain without symptoms or signs for periods of weeks or even months\(^{19,20}\). Further study is needed to manage infection in the pregnant uterine cavity in the perinatal period.

In summary, the present study shows that absent PGF\(_2\alpha\)\(^{21}\), attenuated cortisol and fetal cardiovascular responses to high-dose endotoxin administration to the fetus. These results suggest that fetal absent or attenuated responses, relative to the adult, may be a self-protective mechanism that diminishes premature delivery and fetal asphyxia.

Further study is needed to clarify the mechanism of the absence or attenuation of the response to endotoxin in the fetus.

References


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