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Changes in Fetal Plasma Adenosine and Xanthine Concentrations during Fetal Asphyxia with Maternal Oxygen Administration in Ewes

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Suzuki, S., Yoneyama, Y., Sawa, R., Murata, T., Araki, T. and Power, G. G. *Changes in Fetal Plasma Adenosine and Xanthine Concentrations during Fetal Asphyxia with Maternal Oxygen Administration in Ewes.* Tohoku J. Exp. Med., 2000, **192** (4), 275-281 —— In this study, we measured fetal plasma adenosine and xanthine concentrations during and after severe asphyxia, and investigated the key issues related to oxygen therapy. Asphyxia was induced by occluding the umbilical cord for 5 minutes in 6 fetal sheep with and without the administration of oxygen to the ewe. Plasma adenosine concentration increased significantly during cord occlusion in the all fetuses, and the differences between the values in the fetuses with and without maternal oxygen administration was not significant. By 30 minutes after cord release, plasma adenosine concentration in all fetuses had returned to levels similar to those at the start of the experiment. Plasma xanthine concentration also increased during cord occlusion in all fetuses. However, 30 minutes after cord release, plasma xanthine concentration had decreased significantly in fetuses without additional oxygen, while it did not change significantly in fetuses with maternal oxygen administration. Thus, we speculated that maternal oxygen administration before fetal asphyxia may not contribute to additional ATP stores in fetal organs and may produce oxygen free radicals following asphyxia. ———— adenosine; xanthine; asphyxia; fetal sheep; oxygen therapy

Oxygen administration to the mother is a common therapy for fetal asphyxia. In earlier studies, maternal oxygen administration was reported to increase the fetal reduced PaO_2 to normal and stimulate fetal breathing movements in hypoxemic fetuses (Boddy et al. 1974; Nicolaides et al. 1987).

On the other hand, the release of oxygen free radicals has been investigated during the phase of re-oxygenation (Kjellimer et al. 1989). During asphyxia,

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adenosine and hypoxanthine were degraded from adenosine triphosphate (ATP) (Mishra and Delitoria-Paradopoulos 1989). Hypoxanthine is further metabolized to xanthine by xanthine oxidase, which is an important souce of harmful oxygen free radicals following asphyxia (Fridovich 1970). Thus, a large quantity of xanthine oxidase and xanthine may be released into the fetal circulation with the administration of maternal oxygen administration following asphyxia.

In this study, we measured the changes in fetal plasma adenosine, hypoxanthine and xanthine concentrations during and after severe asphyxia via an umbilical cord occlusion with and without giving oxygen to the ewe. We also investigated the key issues related to oxygen therapy.

Materials and Methods

The study was approved by the Loma Linda University Animal Use Committee. Six pregnant ewes were operated on 126–134 days of gestation (term = 147 days) using the method previously reported (Kubonoya and Power 1997). Briefly, anesthesia was induced with thiopental (10 mg/kg, i.v.) and maintained with 2% halothane in oxygen. Under sterile conditions, incisions were made in the maternal skin and uterine wall, and the fetal head and upper torso were partially delivered. An inflatable silicon cuff was placed loosely around the umbilical cord. A polyvinyl catheter (1.8 mm outside diameter) was placed in the right carotid artery, and its tip was advanced to the ascending aorta for blood sampling and pressure recording. An additional catheter (2.2 mm outside diameter) was placed in the amniotic cavity. The uterine wall was closed. The catheters were brought to the surface via an incision and protected using a nylon pouch. The sheep was allowed to recover from surgery for at least 72 hours before experiments were begun. Ampicillin, 500 mg, and gentacin, 40 mg, were instilled into the amniotic cavity, and penicillin G, 4×10^5 units, was administrated to the ewe daily.

Two similar experiments were performed in each sheep, separated by a period of 48 hours. In control experiments, the umbilical cord was occluded promptly for 5 minutes using 5–8 ml sterine saline in an inflatable cuff after an initial 30-minute control period. The cord was then released and the fetal responses were followed during a 30-minute recovery period. This single occlusion/release method was chosen because it was shown previously (Suzuki and Power 1999) to cause marked hypoxemia and a detectable increase in plasma adenosine in fetal sheep, but is not so severe as to cause brain damage (Myers 1972).

Experiments with giving 100% oxygen to the ewe began with the initial 30-minute control period. The administration of oxygen was done by passing 30 liter/minutes O_2 through a plastic bag over the ewe's head. In our pilot study ($n = 2$), maternal PaO_2 levels were increased approximately from 95 to 180–250 mmHg using this method. Twenty minutes after, the umbilical cord was occluded for 5 minutes.

Throughout the experiments, fetal blood pressure and heart rate were recorded continuously (Gould, Model 200, Valley View, OH, USA). Fetal arterial blood samples (1.0 ml) were collected 30 minutes before the cord occlusion at the beginning of the onset of cord occlusion, and at −1, 5, 20 and 35 minutes. The first 0.5 ml of the blood sample was withdrawn into a heparinized syringe and was immediately added to an equal volume of ice-cold stop solution (9-erythro-2-[hydroxy-3-nonyl] adenine, 120 μM; dipyridamole, 20 mM; α, β methylene adenosine-5′-diphshate, 60 mM; and ethylenediamineteraacetic acid dipotassium salt, 4.4 mM) for measurement of plasma adenosine, hypoxanthine and xanthine concentrations. The mixtures were then centrifuged at 3000 rpm for 5 minutes at 4°C. The plasma was transferred to an ultrafiltration cone (Amicon; Millipore Co., Bedford, MA, USA) and deproteinized by centrifugation at 6000 rpm for 1 hour at 20°C. Samples of ultrafiltrate were stored at −70°C until analysis using high-performamnc liquid chromatography using the method previously reported (Wynants and Van Belle 1985; Kubonoya and Power 1997). Briefly, 50 μl of the ultrafiltrates was injected into a C18 column (Radial-Pac; Waters, Milford, MA, USA) and the absorbance of the eluate was monitored continuously at 254 nm for purine activity. The second 0.5 ml of the blood sample was used for measurement of blood gas (ABL3, Radiometer, Copenhagen, Denmark).

After the experiments, the ewe and fetus were sacrificed and the fetus weight was recorded to the nearest gram.

The results are expressed as mean±S.E.M. Significant differences ($p<0.05$) were determined by one-way analysis of variance ANOVA and Fisher's least significant difference for multiple comparisons test, and paired and unpaired t-test.

Results

Six fetal sheep, with a mean weight of 3.4±0.3 kg, were studied on 129–141 days of gestation.

During umbilical cord occlusion, the fetal mean blood pressure increased and the heart rate decreased significantly in both the control and oxygen experiments (Fig. 1). These changes were observed as marked variable decelerations. There were no significant differences in the time course of responses after the administration of maternal oxygen.

Table 1 shows the changes in fetal arterial pH, PaO_2 and $PaCO_2$ in the both experiments. Twenty minutes after the start of maternal oxygen administration, fetal PaO_2 increased from 21.4±2.1 to 27.6±1.3 ($p<0.05$ by the paired t-test), while there was no significant change in fetal arterial pH or $PaCO_2$. However, during the occlusion periods, the degrees of hypoxemia and acidemia were not altered by prior maternal administration of oxygen. During the recovery, there was a significant difference in fetal PaO_2 between the two groups ($p<0.05$).

Fig. 2 shows the changes in plasma adenosine, hypoxanthine and xanthine concentrations under both conditions. Plasma adenosine and hypoxanthine

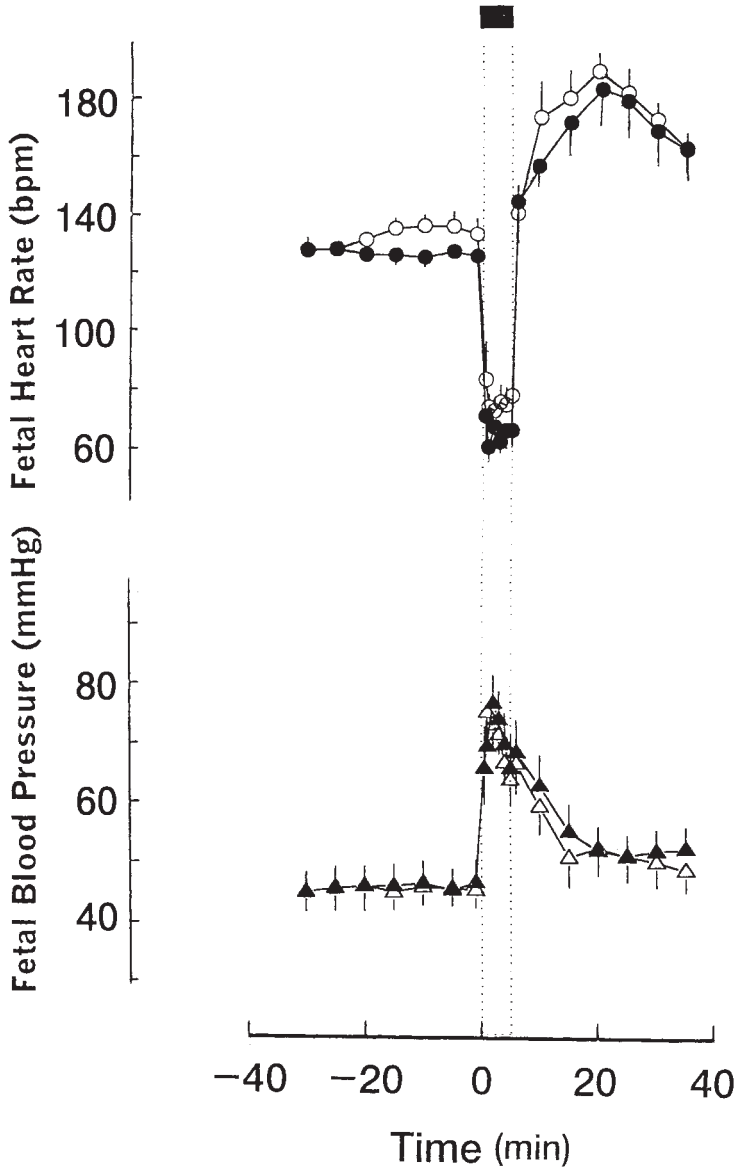

Fig. 1. Changes in fetal heart rate and mean blood pressure responses to 5-minutes umbilical cord occlusion with (open circles and triangles) and without (closed circles and triangles) oxygen administration to the ewe ($n=6$). Period of umbilical cord occlusion is shown by closed bar (■).

concentrations increased significantly during cord occlusion in all the fetuses, and the differences between the values in the fetuses with and without maternal oxygen administration was not significant ($p<0.05$ by ANOVA). By 30 minutes after cord release, plasma adenosine and hypoxanthine in all fetuses had returned to levels similar to those at the start of the experiments.

Plasma xanthine concentration also increased during cord occlusion all fetuses ($p<0.05$). Thirty minutes after cord release, plasma xanthine concentration had decreased significantly in fetuses without maternal oxygen administration ($p<0.05$ by ANOVA), while it did not change significantly in fetuses with maternal oxygen administration. At this time, there were also significant differences in plasma xanthine levels between the two groups ($p<0.05$ by the unpaired t-test).

TABLE 1. *Changes in fetal arterial pH, $PaCO_2$ and PaO_2 responses to 5-minute umbilical cord occlusion with and without oxygen administration to the ewe ($n=6$)*

Time interval (min)	Control		Occlusion	Recovery	
	−30	−1	5	20	35
Without maternal oxygen administration					
pH	7.35±0.03	7.35±0.01	7.07±0.05	7.21±0.09	7.26±0.02
$PaCO_2$ (mmHg)	46.5±2.1	47.1±2.0	87.0±6.0	53.4±2.1	50.0±3.5
PaO_2 (mmHg)	21.0±0.81	20.5±0.72	5.9±1.5	19.9±0.93	22.5±1.8
With maternal oxygen administration					
pH	7.35±0.03	7.31±0.03	7.06±0.08	7.20±0.06	7.27±0.03
$PaCO_2$ (mmHg)	45.5±2.4	49.0±3.3	77.5±11	54.5±4.4	51.4±2.5
PaO_2 (mmHg)	21.4±2.1	27.6±1.3*	6.0±1.8	26.6±3.1*	28.7±2.7*

* Significantly different from the group without oxygen administration, by unpaired t-test ($p<0.05$).

DISCUSSION

During reduced oxygenation and ischemia, fetal adenosine is degraded from ATP (Mishra and Delitoria-Paradopoulos 1989). Adenosine is a vasoactive purine metabolite that is a powerful vasodilator in many organs and a suppressor of metabolic processes (Ohisalo 1987). Hypoxanthine is also an intermediate break-down product of ATP (Mishra and Delitoria-Paradopoulos 1989), and its concentration in plasma has been investigated as an indicator of the degree of tissue hypoxia (Thiringer et al. 1981; Kjellimer et al. 1989). In this study, there were no significant differences in the levels of plasma adenosine and hypoxanthine during hypoxia or the recovery period between the fetuses with and without maternal oxygen administration, although the fetuses with oxygen therapy may have accumulated a surplus of additional ATP stores. In an earlier study with humans (Polvi et al. 1995), maternal hyperoxydation did not induce any adverse effects in healthy fetuses. Thus, our results suggest that maternal oxygen administration before hypoxia does not contribute to additional ATP stores in fetal organs.

On the other hand, plasma xanthine concentration decreased significantly in fetuses without maternal oxygen administration, while it did not change significantly in fetuses with maternal oxygen administration during the recovery periods, although neither plasma hypoxanthine or xanthine levels were significantly changed during hypoxia. Xanthine oxidase, which is an important souce of harmful oxygen free radicals following asphyxia (Fridovich 1970), is present in the endotherial cells of the whole body, such as brain and heart (Betz 1989; Mishra and Delitoria-Paradopoulos 1999). Fetal reoxygenation by cord release may produce the xanthine oxidase-catalyzed oxidation of hypoxanthine to

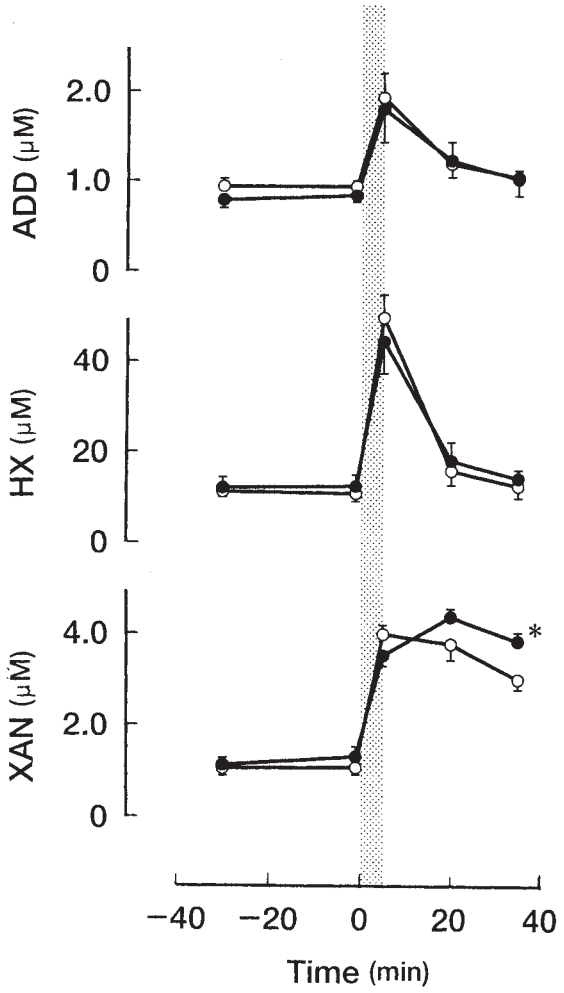

Fig. 2. Changes in fetal plasma adenosine (ADO), hypoxanthine (HX) and xanthine (XAN) concentrations after 5-minute umbilical cord occlusion with (closed circles) and without (open circles) oxygen administration to the ewe ($n=6$). Period of umbilical cord occlusion is shown by shadow. By unpaired t-test, there were significant differences in plasma xanthine levels between the two groups at 35-minutes ($^*p<0.05$).

xanthine and uric acid with the concominant formation of oxygen free radicals, which may cause cellular dysfunction or cell death (Chambers et al. 1985) In this study, we estimated xanthine oxidase activity by measurring the amount of xanthine produced from hypoxanthine. However, histological changes in fetal brain or heart following asphyxia and the changes in blood flow to organs were not measured in this study. Acute asphyxia has been reported to reflect fetal circulatory balance between central and peripheral organs (Jensen et al. 1987), and this re-distribution causes the change in oxygen consumption in each organ during asphyxia. In sheep (Blanco et al. 1988), in addition, an increase in fetal PaO_2 above normal has been also observed to decrease in the organ blood flow to the fetal brain, coronary and placenta. From our results, we speculate that a larger quantity of xanthine oxidase was released into the fetal systemic circulation after cord release in fetuses with maternal oxygen administration than in untreated

fetuses.

In summary, maternal oxygen administration before fetal asphyxia may not contribute to additional ATP stores in fetal organs and may produce oxygen free radicals following asphyxia. Thus, we suggest that oxygen therapy may be inadequately for management of fetuses with variable decelerations.

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