BASIC SCIENCE: OBSTETRICS

Systemic and cerebral inflammatory response to umbilical cord occlusions with worsening acidosis in the ovine fetus

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OBJECTIVE: We hypothesized that repetitive umbilical cord occlusions (UCOs) with worsening acidosis will lead to a fetal inflammatory response.

STUDY DESIGN: Chronically instrumented fetal sheep underwent a series of UCOs until fetal arterial pH decreased to <7.00. Maternal and fetal blood samples were taken for blood gases/pH and plasma interleukin (IL)-1B and IL-6 levels. Animals were euthanized at 24 hours of recovery with brain tissue processed for subsequent measurement of microglia and mast cell counts.

RESULTS: Repetitive UCOs resulted in a severe degree of fetal acidemia. Fetal plasma IL-1B values were increased ~2-fold when measured at maximal fetal acidosis and again at 1-2 hours of recovery. Fetal microglia cells were increased ~2-fold in the white matter and hippocampus, while mast cells were increased ~2-fold in the choroid plexus and now evident in the thalamus when analyzed at 24 hours recovery.

CONCLUSION: Repetitive UCOs leading to severe acidemia in the ovine fetus near term will result in an inflammatory response both systemically and locally within the brain.

Key words: fetal hypoxia, interleukin-IB, interleukin-6, mast cells, microglia

Cite this article as: Prout AP, Frasch MG, Veldhuizen RAW, et al. Systemic and cerebral inflammatory response to umbilical cord occlusions with worsening acidosis in the ovine fetus. Am J Obstet Gynecol 2010;202:82.e1-9.

Birth asphyxia with severe fetal acidemia, defined as an umbilical artery pH <7.00, is associated with increased risk for newborn hypoxic-ischemic encephalopathy (HIE), although the majority of these infants will still be without noted complications.[1-4] This indicates that birth asphyxia with resultant brain injury in most instances is multifactorial in basis with gestational age at birth, duration of hypoxic acidemia, fetal/newborn compensatory capacity, and newborn resuscitation also likely to be contributory.[3,5,6]

There is now considerable epidemiologic and clinical evidence that increases in inflammatory cytokines during the course of infection play a contributing role in the increased risk for brain injury, whether intrauterine with chorioamnionitis preterm or at term, or postnatal in the neonate.[7] This has resulted in a number of animal-based studies with the induction of perinatal infection and/or inflammatory response by bacterial products further implicating a contributory role for an increase in fetal inflammatory cytokines along with an increase in inflammatory cells within the brain, in resultant brain injury.[7-9] These animal-based studies furthermore show an interactive effect whereby bacterial endotoxin sensitizes the immature brain to hypoxic-ischemic injury indicating that infection and hypoxic acidemia may have a synergistic role in causing fetal brain injury.[7,10,11] There is also considerable clinical and experimental evidence that increases in inflammatory mediators play a contributory role in the pathogenesis of newborn HIE in the absence of overt infection, although most cases of histopathologic chorioamnionitis will be subclinical especially at term.[7,12-14] In addition, hypoxia and hypoperfusion both lead to increases in cytokine expression and/or production within the placenta[15,16] supporting the contention that reduced uterine or umbilical blood flow with contractions through labor might lead to an increase in inflammatory cytokines as well as worsening fetal acidosis.

Variable-type fetal heart rate (FHR) decelerations due to umbilical cord compression with acute reduction in fetal oxygenation are the most common nonreassuring FHR pattern observed intrapartum.[17] Although these short-term hypoxic episodes are generally well tolerated, when more frequent and/or severe they have been associated with an increased incidence of neonatal acidosis, low Apgar scores, and nuchal cord involvement at the time of delivery.[18,19] We have therefore used the chronically catheterized ovine fetus near term to test

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Received Jan. 28, 2009; revised May 29, 2009; accepted Aug. 17, 2009.

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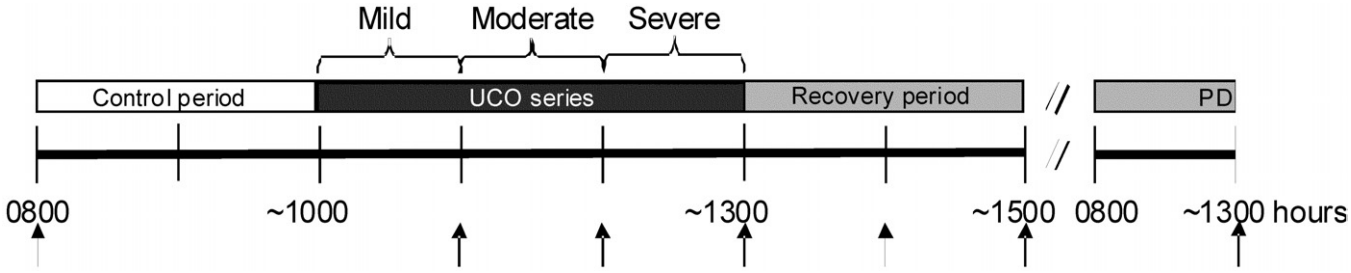
Supported by Grants from the Canadian Institute of Health Research and Children's Health Research Institute London to B.S.R., who holds the Canada Research Chair in Fetal and Neonatal Health and Development.

0002-9378/$36.00

doi: 10.1016/j.ajog.2009.08.020

FIGURE 1
Experimental protocol



Animals were studied through 1- to 2-hour control period; ~3 hours of mild, moderate, and severe repetitive umbilical cord occlusions (UCOs); and then recovered for ~24 hours. *Arrows* indicate 3-mL fetal and/or maternal blood samples.

PD, putdown.

Prout. Inflammation with fetal asphyxia. Am J Obstet Gynecol 2010.

the hypothesis that repetitive cord occlusions with worsening acidosis as might be seen clinically during labor will lead to an inflammatory response both systemically and locally within the brain. The proinflammatory cytokines interleukin (IL)-1B and IL-6 have been determined as measures of systemic inflammation because these cytokines play a prominent regulatory role in the inflammatory response and have been shown to increase as part of the fetal/neonatal inflammatory response to infection and with HIE.[7,12-14] The distribution of microglia and mast cells within the brain has been determined as a measure of local inflammation because these cells also play a prominent role in the inflammatory response and likewise have been shown to increase with fetal/neonatal infection and/or hypoxia.[7-9,20]

MATERIALS AND METHODS

Surgical preparation

Ten near-term (125 ± 1 days' gestation) fetal sheep of mixed breed were surgically instrumented (term = 145 days). The anesthetic and surgical procedures and postoperative care of the animals have previously been described.[21] Briefly, using sterile technique under general anesthesia, the upper body of the fetus and proximal portion of the umbilical cord were exteriorized through an incision in the uterine wall. Polyvinyl catheters (Bolab, Lake Havasu City, AZ) were placed in the right and left brachiocephalic arteries, and the right brachiocephalic vein. Stainless steel electrodes were implanted biparietally on the dura for the recording of electrocortical activity and over the sternum for recording electrocardiographic (ECG) activity. An inflatable silicone occluder cuff (OCHD16; In Vivo Metric, Healdsburg, CA) was positioned around the proximal portion of the umbilical cord and secured to the abdominal skin. Once the fetus was returned to the uterus, a catheter was placed in the amniotic fluid cavity and subsequently in the maternal femoral vein.

Animals were allowed a 3- to 4-day postoperative period before experimentation, during which antibiotics were given and catheters were flushed with heparinized saline to maintain patency. Animal care followed the guidelines of the Canadian Council on Animal Care and was approved by the University of Western Ontario Council on Animal Care.

Experimental protocol

Animals were studied through a 1- to 2-hour control period and an experimental period of repetitive umbilical cord occlusions (UCOs) with worsening acidemia, and were then allowed to recover overnight (Figure 1). A computerized data acquisition system was used to record pressures in the fetal brachiocephalic artery and amniotic cavity, and the electrical signals for electrocortical and ECG activities, which were monitored continuously through the control and experimental periods, and first 2 hours of the recovery period (Chart 5 for Windows; AD Instruments Pty Ltd, Castle Hill, Australia).

After the baseline control period that began at ~0800 hours, repetitive UCOs were performed with increasing severity until severe fetal acidemia was detected (arterial pH <7.00), at which time the UCOs were stopped. UCO was induced by complete inflation of the occluder cuff with ~5 mL of saline solution that was previously determined by visual inspection and testing at the time of surgery. During the first hour a mild UCO series was performed consisting of cord occlusion for 1-minute duration every 5 minutes. During the second hour a moderate UCO series was performed consisting of cord occlusion for 1-minute duration every 3 minutes. During the third hour a severe UCO series was performed consisting of cord occlusion for 1-minute duration every 2 minutes and this series was continued until the targeted fetal arterial pH was attained. Following the mild and moderate UCO series a 5- to 10-minute period with no UCO was undertaken, during which fetal arterial blood was sampled and arterial blood pressure, electrocortical, and ECG data were recorded in the absence of FHR decelerations. After attaining the targeted fetal arterial pH <7.00 and stopping the repetitive UCOs, animals were allowed to recover for ~24 hours.

Fetal arterial blood samples were obtained during the baseline control period (3 mL), at the end of the first UCO of each UCO series (1 mL), and ~5 minutes after each UCO series (3 mL). In ad-