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Research Paper

Preterm white matter brain injury is prevented by early administration of umbilical cord blood cells

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ABSTRACT

Infants born very preterm are at high risk for neurological deficits including cerebral palsy. In this study we assessed the neuroprotective effects of umbilical cord blood cells (UCBCs) and optimal administration timing in a fetal sheep model of preterm brain injury. 50 million allogeneic UCBCs were intravenously administered to fetal sheep (0.7 gestation) at 12 h or 5 d after acute hypoxia-ischemia (HI) induced by umbilical cord occlusion. The fetal brains were collected at 10 d after HI. HI ($n = 7$) was associated with reduced number of oligodendrocytes (Olig2+) and myelin density (CNPase+), and increased density of activated microglia (Iba-1+) in cerebral white matter compared to control fetuses ($P < 0.05$). UCBCs administered at 12 h, but not 5 d after HI, significantly protected white matter structures and suppressed cerebral inflammation. Activated microglial density showed a correlation with decreasing oligodendrocyte number ($P < 0.001$). HI caused cell death (TUNEL+) in the internal capsule and cell proliferation (Ki-67+) in the subventricular zone compared to control ($P < 0.05$), while UCBCs at 12 h or 5 d ameliorated these effects. Additionally, UCBCs at 12 h induced a significant systemic increase in interleukin-10 at 10 d, and reduced oxidative stress (malondialdehyde) following HI ($P < 0.05$). UCBC administration at 12 h after HI reduces preterm white matter injury, via anti-inflammatory and antioxidant actions.

1. Introduction

Approximately 12% of infants are born preterm, and very preterm infants, born <32 weeks gestational age, are the highest risk group for neurological morbidities. White matter injury (WMI) is the most common brain damage in preterm infants, typically localized to the periventricular white matter (PVWM) area in a diffuse or focal pattern (Woodward et al., 2006). In all preterm infants with WMI, 5–15% will develop cerebral palsy, and 50% will have cognitive, behavioral or attention deficits (Volpe, 2003). Cerebral ischemia-reperfusion and inflammation are the two principal causes of WMI (Dammann and Leviton, 1997; Buser et al., 2012; Back et al., 2007), leading to neuropathologies that include disruption to oligodendrocyte development, deficits in axonal myelination, astrogliosis, and/or microglial activation (Khwaja and Volpe, 2008). Oligodendrocyte progenitor cells present between 23 and 32 weeks gestation in humans are particularly susceptible to inflammation and/or hypoxia-ischemia (Back and Volpe, 1997). The complexity of white matter neuropathology, relative brain immaturity and inherent susceptibility of very preterm infants means that there are currently no neuroprotective treatments available for this vulnerable cohort.

Umbilical cord blood contains a diverse and rich mix of stem and progenitor cells with excellent potential for neurorepair, and is readily available (Castillo-Melendez et al., 2013; Li et al., 2014; Bennet et al., 2012). There is increasing evidence to demonstrate the beneficial effects of UCBCs in preventing or repairing HI-induced brain injury in term-equivalent newborn rats (postnatal day 7–10), when xenotransplanted human UCBCs are administered within 24 h after HI (Li et al., 2014; Bennet et al., 2012; Geissler, 2011; Pimentel-Coelho et al., 2010; Meier et al., 2006; Wasielewski et al., 2012). Human UCBCs transplanted intraperitoneally decrease apoptotic and necrotic cell death within the brain, mediated by reduced brain inflammation (Pimentel-Coelho et al., 2010; Geissler et al., 2011). While the white to gray matter ratio and pattern of brain injury is different in young rats (Rees and Inder, 2005), one study in preterm-equivalent (postnatal) rats supports that intravenous human UCBC administration may preserve white matter architecture following HI (Hall et al., 2009).

We tested the hypothesis that intravenously administered allogeneic UCBCs would protect the developing white matter of the preterm

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sheep brain following HI. We examined whether early (12 h) or late (5 d) administration of UCBCs after insult would have differential effects, and explored the mechanisms of UCBC action. UCBCs prevent neuronal degeneration when administered 12 h after birth asphyxia in term lambs (Aridas et al., 2015), but their role in white matter protection is not known, and this early administration may be unrealistic in clinical practice for brain injury in very preterm infants. Therefore we have selected a comparison between UCBC administration at 12 h versus 5 d. Prolonged umbilical cord occlusion (UCO) in preterm fetal sheep at 0.65–0.7 gestation induces significant WMI mediated by hypoxemia, inflammation, excitotoxicity, and oxidative stress (Bennet et al., 2007; Ferreiro, 2006). Accordingly, we induced a period of severe HI in preterm fetal sheep at a time corresponding to 28–32 weeks gestation of human white matter development (Bennet et al., 2007), to examine the neuroprotective benefits and preferred timing of UCBC therapy.

2. Materials and methods

2.1. Animals and surgery

Surgery was performed on 38 pregnant Merino-Border Leicester ewes of known gestational age bearing single (n = 34) and twin fetuses (n = 8) at 97.5 ± 0.1 d gestation (term = ~147 d). All surgical and experimental procedures were approved by the Monash Medical Centre Animal Ethics Committee (MMCA/2013/17). Anesthesia was induced with intravenous (iv) 20 mg/kg sodium thiopentone (Bomac Laboratories, New Zealand) and maintained with 2% isoflurane (Abott, Australia) via an endotracheal tube. Under aseptic conditions, the ewe underwent a laparotomy to exteriorize the fetus, and polyvinyl catheters (0.8 mm inner diameter, 1.2 mm outer diameter, Dural Plastics, Australia) were inserted into the fetal femoral artery for monitoring arterial blood pressure and obtaining blood samples, and fetal vein for administering UCBCs or saline. An inflatable balloon occluder (16HD, In Vivo Medical, USA) was placed around the umbilical cord to induce HI. The fetus was returned to the uterus, and the uterine and abdominal incisions sutured in layers. All catheters were exteriorized through the maternal flank, and the muscle layers closed separately. A maternal jugular vein catheter was also implanted for antibiotic administration. Prior to surgery and for 3 days post-surgery, 500 mg engemycin (Coopers, Bendigo East, Australia) and 1 g ampicillin (Lennon Healthcare, St Leonards, Australia) were administered iv to the ewe.

Experiments were conducted 4–5 d postoperatively. Fetal catheters were maintained by continuous infusion of heparinized saline (50 IU/ml, 0.2 ml/h). Fetal heart rate and mean arterial pressure (MAP), corrected for amniotic fluid pressure, were monitored using pressure transducers during the experiment, and digitized and stored for off-line analysis (Power Lab, AD Instruments, Castle Hill, Australia).

2.2. Experiment protocol (Fig. 1A)

At 102.3 ± 0.2d gestation (0.7 gestation), animals were randomized into one of five groups: (1) control (sham-occlusion + iv saline, n = 10); (2) HI (HI + saline, n = 7); (3) HI + UCB@12 h (HI + 50 million UCBCs injected iv at 12 h after UCO, n = 6); (4) HI + UCB@5 d (HI + 50 million UCBCs at 120 h, n = 6); (5) control + UCB@12 h (sham-occlusion + 50 million UCBCs at 12 h, n = 5). The HI + UCB@12 h, HI + UCB@5 d and control + UCB@12 h groups used singleton fetuses only, while the HI and the control group used singletons and twins, and there was no difference in sex distribution (Table 1). HI was achieved by complete UCO, in which the balloon occluder was filled with 2.0–2.5 ml water for 23-25 min. The occlusion was discontinued at 25 min or sooner if the occlusion was >23 min duration and MAP had decreased to <8 mm Hg. Fetal arterial blood samples (approximately 0.5–1.5 ml) were collected 24 h before, during, and 6, 12, 24, 48, 72, 120, and 240 h after HI for blood gas parameters (ABL 700, Radiometer, Copenhagen, Denmark), cytokine and malondialdehyde concentrations. Plasma samples were stored at −80 °C until assays were performed.

2.3. Cell preparation

Umbilical cord blood was collected at cesarean-section of near-term lambs (141 d gestation). The umbilical cord was clamped and cord blood from the placental side was collected into heparinized syringes. UCBCs were isolated by centrifuging the blood at 3100 rpm for 12 min at room temperature, without brake. The buffy coat layer was collected, excess red blood cells were removed using red blood cell lysis buffer (155 mM NH4Cl, 10 mM $KHCO_3$, 0.1 mM EDTA in H_2O). The cells were resuspended in bovine fetal serum with 10% DMSO (Merck, Darmstadt, Germany), and cryopreserved in liquid nitrogen. The cells were thawed just prior to administration. Cell yield and viability were assessed using the Trypan blue dye exclusion method. 50 million viable cells were labeled with carboxyfluorescein succinimidyl ester (CFSE) to enable tracking of the cells within the brain (Yawno et al., 2013). Cells were suspended in 2.5 ml sterile saline, and administered to the fetus (over 3 min) via the femoral vein.

2.4. Tissue collection and processing

At 10 d after UCO (112.5 ± 0.2 d gestation), the ewe and fetus were euthanized by iv overdose of sodium pentobarbital (Virbac, Peakhurst, Australia) to the ewe. The fetus was removed and fetal body weight

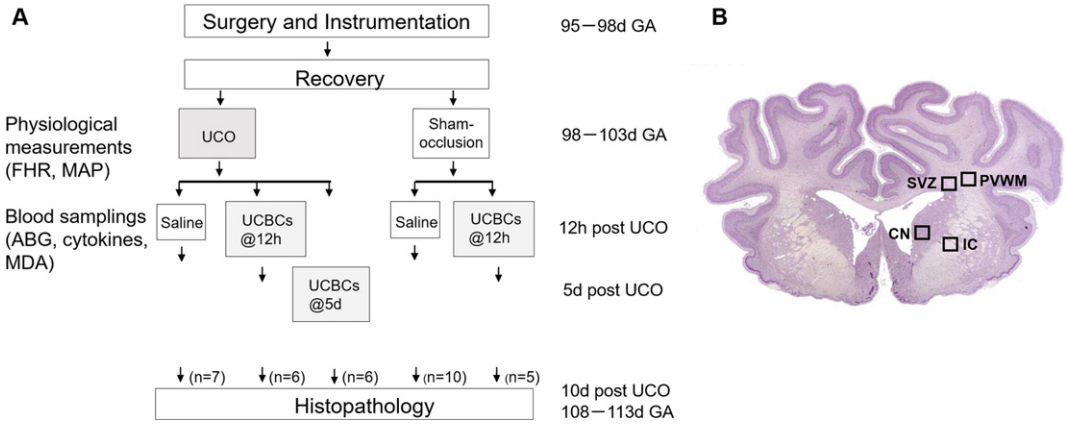

Fig. 1. A: Experimental timeline. ABG: arterial blood gas; FHR: fetal heart rate; GA: gestational age; HI: hypoxia-ischemia; MAP: mean arterial pressure; MDA: malondialdehyde; UCBCs: umbilical cord blood cells; UCO: umbilical cord occlusion. B: Representative lamb brain photomicrograph of anatomical regions assessed for histology (Sheep Ovis aries–Section 720). SVZ: subventricular zone; PVWM: periventricular white matter; IC: internal capsule; CN: caudate nucleus.