Human amniotic epithelial cell therapy for preterm perinatal asphyxia

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Background. Premature infants are at significant risk of asphyxia before birth and consequent brain injury. About half survive with significant neurological and cognitive disability and approximately 10 to 15% develop cerebral palsy. For term infants, therapeutic hypothermia is now well established as a therapy for hypoxia ischemia (HI), however in preterm neonates this therapy has not been as successful. Studies performed here in preterm fetal sheep suggest that while hypothermia may improve recovery it also reduces the proliferation and restoration of oligodendrocytes causing white matter deformations and subsequently also gray matter abnormalities. Thus, it is critical to further neuroprotection treatments and to actively promote brain recovery b by production of new brain cells.

One potential therapeutic intervention is the administration of human amniotic epithelial cells (hAECs) to not only reduce damage caused, but also to repair damage that has already been established. The amniotic membrane arises approximately 8 days after fertilization from the epiblast making it the earliest fetal tissue to separate from the embryo proper. This allows hAECs to differentiate into cells of all three germ layers, even when harvested from term amniotic membranes. Stem cell transplantation studies performed in adult models of brain damage have shown promising results in diminishing lesion size and improving functional outcome *via* multiple pathways. First, it has been shown that hAECs can differentiate into neural progenitors and can survive *in vivo* for up to 2.5 months. Secondly, hAECs are able to modulate the immune response post ischemia and hereby may reduce secondary damage caused by inflammation. Thirdly, hAECs produce neurotrophic factors that stimulate survival, growth and differentiation of host neural stem cells. In addition hAECs do not induce an immune response as there is very limited MHC expression. Thus hAECs treatment may improve outcome for premature infants that suffered from intra-uterine hypoxia ischemia reducing morbidity and mortality. We hypothesize that administration of hAECs will decrease brain injury and improve functional recovery by reducing damage caused by chronic inflammation, supporting host progenitor cell proliferation and differentiation and cell replacement.

Aim. To test whether hAECs can reduce preterm fetal brain injury after asphyxia *in utero*.

Methods. Ewes were induced with i.v. administration of propofol 2% (5mg/kg) followed by continuous administration of 2-5% isofluorane. The fetus was exposed through a small incision in the uterus, instrumented with catheters and electrodes and then placed back in the uterus. hAECs or vehicle was administered as a 25 min intracerebroventricularly infusion (million cells/ml) to preterm fetal sheep *in utero* 2 h or 24 h after 25 minutes of complete umbilical cord occlusion, near terminal insult. Fetal physiological parameters were monitored continuously and fetuses were euthanized 7 d post-asphyxia for histological assessment. Cytokine levels and tissue damage will be assessed with ELISA and by immunohistochemistry respectively.

Results. Preliminary data show that hAEC treatment was associated with a return of EEG amplitude and frequency to baseline values, delayed seizures over a suppressed background were observed accompanied by a longer period of cerebral hypoperfusion in the hAEC group when compared to sham controls. Histology and ELISAs are now being undertaken to access damage and inflammation respectively.

Discussion. Sustained EEG suppression is associated with significant injury both experimentally and clinically. Delayed hAEC treatment markedly restores EEG activity suggesting that it has reduced injury. These data support the hypothesis that hAECS reduce brain injury in preterm fetal sheep following asphyxia.