

A new approach to modelling infection-induced inflammation and brain injury using bacterial extracellular vesicles in preterm fetal sheep

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Background: Perinatal infection increases the risk of brain injury. Preclinical models of lipopolysaccharide (LPS)-induced inflammation do not fully reflect the diverse pathophysiological responses to bacterial infections, highlighting the need for more comprehensive translational animal models to identify effective therapeutic targets. We aimed to determine whether *Escherichia coli*-derived bacterial extracellular vesicles (BEVs) activate additional inflammatory mechanisms, independent of LPS, more comparable to a live bacterial infection.

Method: Chronically instrumented preterm fetal sheep (102±1 day gestation (d)) were randomised to receive intravenous (i.v.) saline (n=8), repeated, escalating LPS infusions (0 h=100ng, 24 h=200ng, 48 h=400ng; n=8; i.v.) or repeated, escalating BEV infusions (0 h=96EU, 24 h=192EU, 48 h=384EU; n=7, i.v.). Physiological parameters were continuously monitored. Post-mortem was performed at 114±1d, and brains isolated for histopathological assessment.

Results: BEV and LPS-exposure similarly suppressed EEG power ($P<0.05$) between 164-264 h. Spectral edge frequency (SEF)-suppression was greater with BEV-exposure compared to LPS ($P<0.05$). Exposure to LPS and BEVs similarly induced higher numbers of microglia and activated, neurotoxic astrocytes (STAT3+) ($P<0.05$ vs control) in the periventricular and intragryal white matter tracts (PVWM and IGWM, respectively). In BEV-exposed fetuses, NOD1+ cells were higher in the PVWM ($P=0.03$ vs LPS) and numbers of total (Olig2+) and mature (CC1+) oligodendrocytes were lower in the PVWM and IGWM tracts ($P<0.05$ vs LPS and control).

Conclusions: BEV-exposure induced physiological and histological neuroinflammation and white matter injury consistent with exposure to live bacterial infection. BEVs may be a more physiologically relevant bacterial mimetic for modelling infection-induced inflammation and perinatal brain injury.