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AMNIOTIC FLUID STEM CELLS DECREASE INTESTINAL PERMEABILITY IN EXPERIMENTAL NECROTIZING ENTEROCOLITIS

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Aim of the Study: Necrotizing enterocolitis (NEC) has been shown to be associated with disruption of intestinal barrier integrity. Amniotic fluid stem cells (AFSC) have been shown to improve survival, clinical status, gut structure and function in experimental NEC. We hypothesize that AFSC could restore intestinal permeability in experimental NEC.

Methods: Following ethical approval (license no. 32238), NEC was induced in 5-day old C57BL/6 mouse pups for 4 days using hypoxia, lipopolysaccharide (4mg/kg) and gavage feeding of hyperosmolar formula (n=10). Breastfed (BF) pups served as controls (n=10). To study the effect of AFSC on NEC, AFSC was administered intraperitoneal on postnatal day 6 and 7 (n=10). Tissue permeability was measured *ex vivo* by Ussing chamber using fresh ileal tissue. Transcellular and paracellular permeability was measured with Horseradish peroxidase 44 kDa (HRP), and fluorescein isothiocyanate-dextran, 4kDa (FD4) respectively. Concentration in the basolateral side was measured and determined by a kinetic enzymatic assay using a micro-plate reader. In addition, tight junction marker Claudin7 was stained by immunofluorescence staining and gene expression was quantified using real time PCR. Data were compared using one-way ANOVA with Bonferroni post-test.

Main Results: NEC was associated with an increase in transcellular intestinal permeability compared with BF (Figure 1A). AFSC significantly decreased both transcellular (BF: 27.2±7.7; NEC: 52.9±15.6; AFSC: 20.6±7.7; p<0.001) and paracellular (BF: 1.8±0.3; NEC: 2.4±0.6; AFSC: 1.7±0.2; p<0.01) permeability in NEC. During NEC, AFSC administration increased both gene (Figure 1B) and protein (Figure 1C) expression of tight junction marker Claudin7 back to control level (BF: 1.0±0.2; NEC: 0.3±0.1; AFSC: 0.9±0.2; p<0.05).

Conclusion: Intestinal epithelium during NEC is damaged as indicated by increased permeability and decreased cell-cell tight junction protein. These alterations can be reversed by administration of AFSC which may prevent bacterial translocation and systemic inflammation/infection in NEC.