

## POSTER SESSION II (CONT.)

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**DEFINING THE ROLE OF PROTEIN KINASE A AND APOPTOSIS IN NECROTIZING ENTEROCOLITIS**

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Necrotizing enterocolitis (NEC) is a deadly intestinal disease that typically affects premature infants. *Cronobacter sakazakii* (CS) has been associated with outbreaks of NEC. We hypothesized that protein kinase A (PKA) mediated signaling may contribute to epithelial cell death in NEC.

**Methods:**

After IRB approval human intestinal segments were obtained from infants undergoing bowel resection for NEC (September 2012-September 2013), NEC strictures, or ostomy closures (controls). Samples were collected in the operating room and preserved in formalin and cryomoulds. Protein was extracted for western blot analysis with antibodies for markers of apoptosis and PKA. Rat intestinal epithelial cells (IEC-6) were grown to 90% confluence and exposed to CS *in vitro*. PKA inhibitors (KT -5720 & SC-3010) were added at doses of 0.1uM, 1uM and 10uM prior to CS infection. IEC-6 cell apoptosis was assayed by western blot analysis of caspases, and by TUNEL staining using the ApoTag red kit. Differences were analysed with students T-test where appropriate.

**Results:**

Activated protein kinase A was identified by Western blot analysis in human specimens with NEC (n=4), as compared with controls (n=6)  $p < 0.005$ . Additionally increased caspase 3 activation was seen in patients with NEC as compared with controls. CS induces caspase 3 activation after 4 hours of co-culture in IEC-6 cells ( $P < 0.005$ ). PKA is present in IEC-6 and appears to be activated by 4 hours of infection with CS. The addition of a PKA inhibitor prior to IEC-6 infection with CS prevents CS-induced apoptosis (Figure 1)  $p < 0.005$ .

**Conclusion:**

We conclude that PKA mediated signaling may play an important role in CS-induced intestinal epithelial apoptosis. The prospect of PKA inhibitors presents an interesting potential therapeutic line of investigation.