TITLE: *E. coli* O157:H7 stimulates Shiga toxin type 2 cytotoxic effects and translocation across human colonic epithelial cell lines

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ABSTRACT:

Gastrointestinal infection with Shiga toxin (Stx2)-producing enterohemorrhagic *Escherichia coli* causes bloody diarrhea, hemorrhagic colitis and hemolytic uremic syndrome (HUS). *E. coli* O157:H7 is the most prevalent serotype associated with HUS and Stx2 is its major virulence factor. However, the mechanisms involved in the pathogenesis of diarrhea mediated by Stx2 and how toxins cross the intestinal epithelium are largely uncharacterized. Our aim was to study the effects of *E. coli* O157:H7 on human colonic epithelial cells to better understand the means by which Stx2 induces diarrhea and translocate the intestinal barrier. We examined cell viability in human intestinal cell lines (HCT-8, Caco-2) after incubation with purified Stx2, *E. coli* O157:H7 strain 125/99 (O157:H7), its mutant lacking *stx2* gene (O157:H7Δstx2) and filtered O157:H7 supernatant. Cells were grown in 96-well culture plate and viability was measured by neutral red uptake after 1, 4 or 24h incubation period under growth-arrested condition. We have also evaluated the translocation of Stx2 across HCT-8 cells cultured as monolayers on Millicell inserts in presence of O157:H7Δstx2 or O157:H7Δstx2-SN. Dextran-FITC was used as an indicator of paracellular permeability and EDTA (1 mM) as a disruptor of tight junctions. Transepithelial electric resistance was monitored daily during the development of cell culture, and after treatments. Collected basal media cytotoxicity was evaluated on Vero cells and Dextran-FITC was measured by fluorometry. A concomitant increase of both parameters was observed in the presence of O157:H7Δstx2 or its corresponding O157:H7Δstx2-SN on the luminal side. Furthermore, the cytotoxic effect induced by O157:H7 on HCT-8 and Caco-2

cell lines was significantly higher than those observed with Stx2 and O157:H7Δstx2 combined. These results indicate that both transcellular and paracellular pathways are implicated on Stx2 translocation, and that direct contact between O157:H7 and intestinal barrier induce an elevated cytotoxic response that suggest an increase on Stx2 production.

Keywords: bloody diarrhea, Shiga toxin, STEC, HCT-8, Caco-2, human intestinal cells.

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