Heat-stable enterotoxin b produced by Escherichia coli induces apoptosis in rat intestinal epithelial cells

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Abstract
A previous study conducted in our laboratory revealed that cultured cells having internalized heat-stable enterotoxin b (STb) displayed apoptotic-like morphology. We therefore investigated if STb induces apoptosis in the IEC-18 cell line (rat ileum epithelial cells) by verifying the activation of caspases-9, -3 and -8 as well as DNA fragmentation of cells treated with purified toxin. We observed activation of caspases-9 and -3 as well as DNA laddering, indicating that STb induces apoptosis in IEC-18 cells.

Keywords : Apoptosis, caspase, DNA fragmentation, Escherichia coli, STb enterotoxin.

Introduction
Heat-stable enterotoxin b (STb) is one of the toxins produced by enterotoxigenic Escherichia coli (ETEC) strains shown to be responsible for the induction of diarrhea and is most commonly associated with post-weaning diarrhea in piglets (Dubreuil, 2008). STb toxin is capable of forming non-specific pores in pig jejunal brush border membrane vesicles (Gonçalves et al., 2007) and of inducing histological damages of the intestine characterized by shortening and atrophy of the villi and thus reduction of the mucosal surface (Rose et al., 1987). These damages have been associated with diminished absorptive ability of the villi and secretion of electrolytes and water during diarrhea.

A previous study conducted in our laboratory demonstrated that cells having internalized STb displayed mitochondrial potential changes and an apoptotic-like morphology (Gonçalves et al., 2009). Indeed, the ability of pore-forming toxins to induce apoptosis is well documented (Braun et al., 2007; Saka et al., 2008; Tran et al., 2011). Apoptosis is a form of programmed cell death characterized by membrane blebbing, chromatin condensation, and DNA fragmentation. Apoptosis can be induced through either an extrinsic or intrinsic pathway.

Extrinsic apoptosis is activated following the interaction of a ligand and a membrane-bound receptor belonging to the Tumor Necrosis Factor Receptor (TNFR) family. This results in the formation of DISC (Death Inducing Signalling Complex) and the activation of caspase-8. Intrinsic apoptosis is the result of intracellular stress causing a change in mitochondrial membrane potential leading to the formation of the apoptosome and the activation of caspase-9. Both caspases-8 and -9 activate caspase-3 which targets downstream substrates leading to DNA fragmentation and eventual cell death.

As STb has been shown to cause apoptotic-like morphology in cultured cells and as rats are used as an animal model for the study of STb, we investigated the ability of STb to induce apoptosis in rat intestinal epithelial cells in culture.

Material and methods
In order to determine if STb induces apoptosis in intestinal epithelial cells of rats, we treated the IEC-18 cell line (rat ileum epithelial cells) with various quantities (nanomole) of purified STb toxin for a period of 24 hours.
Harvested cells were then assessed for caspases activation and DNA fragmentation. The implication of caspases-9, -3 and -8 was verified using fluorescent substrates specific for each of these caspases. Fluorescence emitted from the cleaved substrates was measured with a fluorescence microplate reader at 500 nm. Extracted DNA of toxin-treated IEC-18 cells was migrated on a 1.8% agarose gel and then visualized under a UV lamp at 260 nm. Staurosporine was used at 2 µM (final concentration) as a positive control for apoptosis.

Results

Activation of caspases-9 and -3 (Figure 1) was observed in IEC-18 cells treated with 0.05 and 0.5 nmol of STb, similarly to cells treated with staurosporine, our positive control for apoptosis. As caspases-9 and -8 are initiator caspases of the intrinsic and extrinsic pathways, respectively, the evaluation of their activation allowed us to determine the precise pathway targeted by STb. Activation of caspase-3 was also assessed to ensure the implication of the caspase cascade in STb-mediated apoptosis. The activation of caspase-9 in our study indicates that the intrinsic pathway is targeted by STb. Treatment of cells with the same amount of toxin yielded similar activation levels of either caspase-9 or -3 but not of caspase-8 (Figure 1), a caspase activated when the extrinsic pathway is involved. The induction of apoptosis by STb through the intrinsic pathway is in accordance with other pore-forming toxins (Génestier et al., 2005; Manente et al., 2008).

![Figure 1](image1.png)

**Figure 1.** Activation of caspases-9, -3 and -8 in IEC-18 cells treated with 0.05 and 0.05 nmol of STb toxin. Negative control consisted of untreated cells whereas our positive control consisted of cells treated with staurosporine (STS). Mean ± standard error of the mean of 2 experiments.

Extracted DNA from IEC-18 cells treated with 0.25 and 0.5 nmol of STb revealed a similar laddering pattern as observed with extracted DNA of cells treated with staurosporine (Figure 2). DNA fragmentation is the result of cleavage by endonucleases of internucleosomal DNA into 180 bp – 200 bp multiples. The fragments of extracted DNA of cells treated with STb are 1000 bp or smaller. Each ladder step is approximately a multiple of 200 bp, indicating the DNA cleavage at internucleosomal sites.

![Figure 2](image2.png)

**Figure 2.** Extracted DNA of IEC-18 cells treated with STb toxin migrated on an agarose gel. M: molecular markers in base pairs; 1: Untreated cells (negative control); 2 and 3: Cells treated with 0.25 and 0.5 nmol of STb, respectively; 4: Cells treated with staurosporine (positive control for apoptosis).

Conclusion

We showed for the first time that STb enterotoxin induces apoptosis in rat intestinal epithelial cells. The activation of caspase-9 and -3, but not of caspase-8, in our study is an indication of STb-mediated intrinsic
apoptosis in IEC-18 cells. This pathway involves a change in mitochondrial membrane potential as it was observed in a previous study conducted in our laboratory but using the NIH-3T3 cell line. DNA fragmentation confirms that apoptosis is occurring in rat intestinal epithelial cells following treatment with STb. Apoptosis of villus epithelial cells can explain, at least in part, the accumulation of fluid observed in pig ligated loops. The death of these cells could be related to a loss of absorptive capacity of the intestine intoxicated by STb toxin.

References