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Full Length Research Paper

Thermostable direct hemolysin of *Vibrio parahaemolyticus* induces morphological changes and disrupts actin in cultured human epithelial cells

Rochman Naim[1,2*] and Fachriyan Hasmi Pasaribu[1]

[1]Department of Animal Diseases and Veterinary Public Health, Faculty of Veterinary Medicine, Institut Pertanian Bogor, Kampus Darmaga, Bogor, Indonesia.
[2]Institute for Research in Molecular Medicine (INFORMM), Universiti Sains Malaysia, Health Campus, 16150 Kubang Kerian, Kelantan, Malaysia.

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The effects of thermostable direct hemolysin (TDH) of *Vibrio parahaemolyticus* on HeLa and Intestine 407 cells were evaluated. The both cells were unexposed and exposed to TDH in the cytotoxic conditions. As assessed by fluorescent actin-staining, TDH-exposed cells showed morphological changes including detachment of cells from their neighbors, apparent loss of cytoplasm with shrinkage of most of the cells. Furthermore, TDH treatment of cells resulted in redistribution of actin with loss of stress fibers, a floccular staining pattern, cellular membrane blebbing, and cell rounding. The actin redistribution was time dependent. We reported here that TDH can induce morphological and cytoskeletal changes in cultured human epithelial cells.

Key words: Thermostable direct hemolysin (TDH), *Vibrio parahaemolyticus*, cultured human epithelial cells, morphological changes, and actin disruption.

INTRODUCTION

Vibrio parahaemolyticus is a major cause of gastroenteritis in areas of the world where seafood is a major part of the diet (Janda et al., 1988). An important virulence factor that has been considered in *V. parahaemolyticus* gastroenteritis is thermostable direct hemolysin (TDH) (Honda and Iida, 1993). TDH is a dimmer composed of two identical subunit molecules of approximately 21,000 Da (Takeda et al., 1978), and genes encoding TDH have been sequenced and cloned (Iida and Yamamoto, 1990).

Cytotoxic effects of TDH have been observed with various cultured cells. After exposure to TDH, morphological damage occurs in cultured mouse myocardial and mouse melanoma cells, including cell shrinking and condensation of the nuclei of both cells (Goshima et al., 1978). In the various eukaryotic cultured cells, TDH induces its cytotoxic effects, and Rat-1 cells were highly sensitive to TDH among 15 cell lines examined (Tang et al., 1997). In our previous studies, TDH induced morphological changes in Rat-1 cells (Naim et al., 2001a; Naim et al., 2001b). TDH also induced apoptosis in Rat-1 cells (Naim et al., 2001b). Most of the studies examining the effects of TDH on cultured cells have used animal cells.

Several studies dealing with TDH on human cultured cells have been reported. TDH was cytotoxic to Intestine 407, a cell line derived from the intestine of human embryos (Tang et al., 1995). TDH in higher concentrations induced cytotoxicity in human Caco-2 intestinal cells (Raimondi et al., 2000). The other study showed the effect of TDH on human erythrocytes (Lang et al., 2004). However, the studies on the effects of TDH directly in cytotoxic conditions on morphology and actin cytoskeleton of cultured human epithelial cells are still not many.

In the present study, we demonstrated the effects of TDH on morphology and actin cytoskeleton of cultured human epithelial cells. We used Intestine 407 and HeLa cell monolayers for visually assessing the effects of TDH

*Corresponding author. E-mail: rochnaim@kb.usm.my. Tel: +60(9)7664956. Fax: +60(9)7672425.

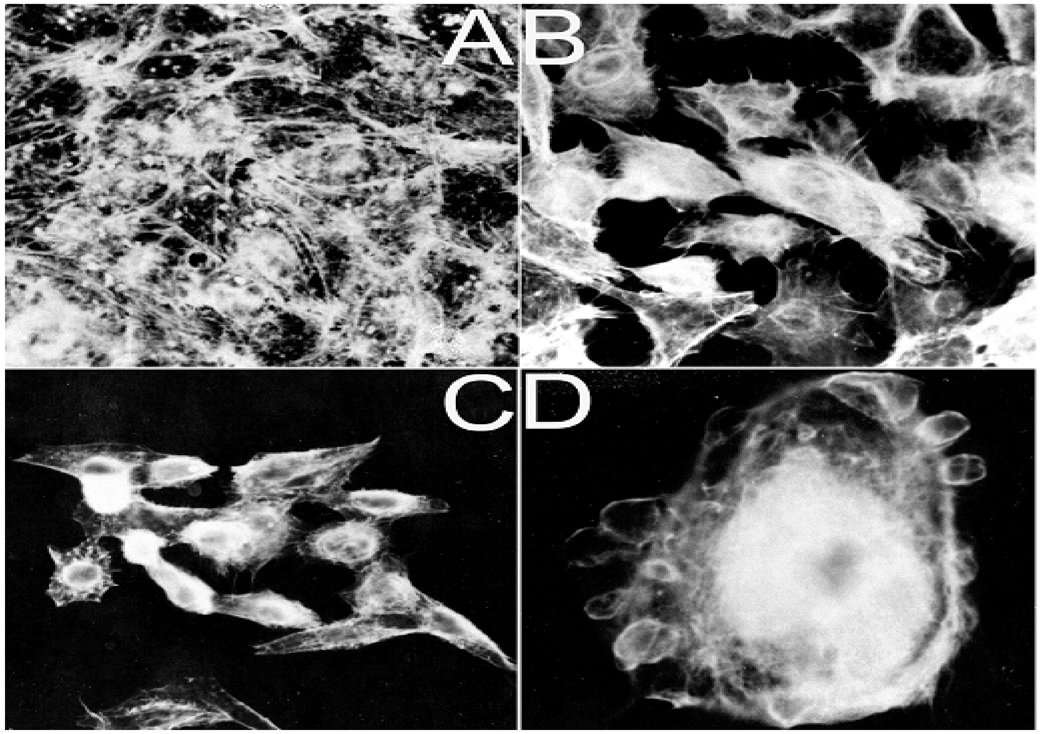

Figure 1. Effects of TDH on the morphology and actin cytoskeleton of HeLa cells**.** Filamentous actin was labeled with rhodamine-phalloidin. Cells were unexposed (A) or exposed for 10 min (B) and 30 min (C and D) to TDH 5 µg/ml at 37°C in DMEM. Magnification is ×400 for A, B and C, and ×1000 for D. Figure 1D shows membrane blebbing of TDH-exposed cells.

on the integrity of epithelial cells, since these two kinds of cells are sensitive to TDH (Tang et al., 1997).

MATERIALS AND METHODS

THD purification

TDH was purified by a previously described method (Tang et al., 1994).

Cell lines and cell culture

Intestine 407 (derived from human intestinal embryonic jejunum and ileum) and HeLa cells (derived from human cervical cancer cells) were grown and maintained in an atmosphere containing 5% CO_2 at 37°C in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% (vol/vol) heat-inactivated fetal bovine serum (FBS), and gentamicin (100 µg/ml).

Morphological studies and actin staining of TDH-exposed cells

Cells grown on glass coverslips in six-well plates until approximately 90% confluent were exposed to TDH in DMEM for 0 - 30 min at 37°C. Cells not treated with TDH served as controls. After treatment, both control and TDH-treated cells were fixed with 3% paraformaldehyde. Then the cells were subjected to actin staining. Actin cytoskeleton of both cells was stained by using rhodamine-phalloidin (Molecular Probes). Images were taken with a fluorescence microscope Olympus BX-50 linked to a camera.

RESULTS

Effects of TDH on morphology of human epithelial cells

We assessed the effects of TDH on epithelial cells, HeLa and Intestine 407 cells, after 30 min of exposure. Untreated control epithelial cells were of uniform size (Figure 1 and 2). Epithelial cells exposed to TDH showed morphological changes including detachment of cells from their neighbors, apparent loss of cell cytoplasm with shrinkage of most of the cells (Figure 1B-1C; Figure 2B-2D).

Effects of TDH on actin cytoskeleton of human epithelial cells

Shrinking of epithelial cells in response to exposure to TDH in this study is thought to be due to disruption of cytoskeletal element, particularly actin. The effects of TDH on actin structure in epithelial cells were assessed after 0-30 min of exposure. Rhodamine-phalloidin stainings of actin of TDH-untreated epithelial cells are shown in Figures 1A and 2A. Fluorescence light microscopy showed actin distributions along the cell surface membrane. These control cells show uniform and organized actin. In contrast, cells treated with TDH revealed distinct separation from each other, loss of stress fibers

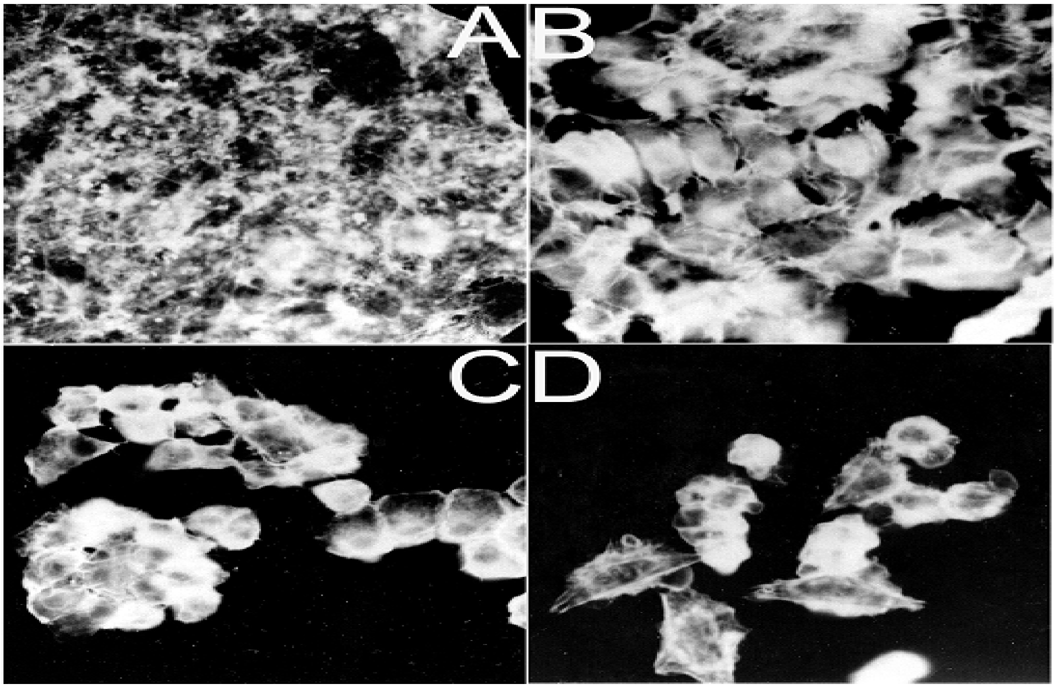

Figure 2. Effects of TDH on the morphology and actin cytoskeleton of Intestine 407 cells**.** Filamentous actin was labeled with rhodamine-phalloidin. Cells were unexposed (A) or exposed for 10 min (B), 20 min (C) and 30 min (D) to TDH 5 μg/ml at 37^{o}C in DMEM. Magnification is ×400. Figure 2D shows membrane blebbing of TDH-exposed cells.

with a floccular pattern of actin staining, and an increased cell globular appearance (Figure 1B-1C; Figure 2B-2D). The small blebs appeared in various regions of the cell border (Figures 1D and 2D). Exposure of epithelial cells to TDH induced a generalized collapse of the actin cytoskeleton to a rounded perinuclear position (Figure 1C-1D; Figure 2C-2D).

The time course of the TDH-exposed HeLa cells is shown in Fig. 1B-1D, and for Intestine 407 cells is shown in Figure 2B-2D. The morphological changes of actin were observed. A rapid and marked disappearance of the actin filaments occurred within 10 min after exposure to TDH (Figure 1B and 2B). The maximal effect evident was detected by 30 min, the actin filaments were no longer seen in the cytoplasm and the cells were totally shrunken and rounded (Figure 1C-1D; Figure 2D).

DISCUSSION

The cellular response to TDH in this study is the dis-assembly of actin microfilaments. The intracellular events that precede actin breakdown are still unknown. The morphological response to TDH consisted of sequential processes which are preceded by alterations of actin organization. The earliest cytoskeletal changes were the disassociation of actin stress fibers from points of attachment in the plasma membrane and loss of visible filament structure (Figures 1B and 2B). This was followed by retraction of actin stress fibers from the periphery to the region surrounding the nucleus and ultimately was associated morphologically with cell rounding (Figure 1C-1D; Figure 2C-2D). These results demonstrate that the breakdown of filamentous actin and shrinkage of the cells associated with TDH cytotoxicity. When the cells were incubated in TDH-free DMEM, no morphological changes of filamentous actin were observed.

In the previous study, we reported that TDH induces apoptosis in Rat-1 cells (Naim et al., 2001a). The active phase of apoptosis is the execution phase which is characterized by the hallmark morphologic features of apoptosis, including membrane blebbing, chromatin condensation, and DNA fragmentation (Mills et al., 1999). The first step in most cells undergoing apoptosis is to partially release extracellular matrix attachments and reorganize focal adhesion, adopting a more rounded morphology. This outward change correlates with loss of actin filaments and a reorganization of actin into a peripheral, membrane-associated ring (Mills et al., 1999). The process is most dramatic in cells that are spread out, with firm matrix attachments and stress fibers, such as fibroblasts (Huot et al., 1998). After the cell rounding up that occurs during release, at the same time, membrane-actin linkages weaken focally, resulting in bleb extrusion in areas of weakness (Mills et al., 1999).

The present study shows that TDH is able to induce morphological and cytoskeletal changes in cultured human epithelial cells in the cytotoxic conditions. Other studies of TDH cytotoxicity on human cultured cells have also been reported. The study of TDH on human Caco-2 cells (Raimondi, 2000) showed that TDH in low concentrations can not cause significant change in the general morphology of the cells. In contrast, TDH at high concentrations induced cell death with significant rounding and detachment of cells. In another study (Tang et al., 1995), TDH caused the damage of plasma membrane and the form of large transparent blebs in Intestine 407. The results of the present study are consistent with these two early studies that TDH induces morphological changes in human cultured cells, including rounding and blebbing of the treated cells.

The present study showed that the morphological changes of TDH-treated cells is related to the disruption of actin skeleton. TDH was also reported to modulate cytoskeletal organization in noncytotoxic conditions, using cultured animal intestinal cells (Fabbri et al., 1999). The results of the present study suggest that TDH may cause disruption of epithelial barrier function in intestine. The loss of actin skeleton may contribute to the diarrhea associated with *V. parahaemolyticus* infection, as well as infections caused by other enteropathogen.

The characteristic of cytotoxicity in response to TDH in this study is the disassembly of filamentous actin. This change is speculated not from direct effects on actin but from the activation of a signal transduction pathway(s) in target cells which still remain to be determined. It cannot be presently excluded the real mechanism of TDH to cause this change. Several mechanisms may underlie the cytotoxicity of TDH in the target cells, and they are needed to be further investigated.

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