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SGLT-1-mediated glucose uptake protects human intestinal epithelial cells against *Giardia duodenalis*-induced apoptosis

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Abstract

Infection with *Giardia duodenalis* is one of the most common causes of waterborne diarrheal disease worldwide. Mechanisms of pathogenesis and host response in giardiasis remain incompletely understood. Previous studies have shown that exposure to *G. duodenalis* products induce apoptosis in enterocytes. We recently discovered that sodium-dependent glucose cotransporter (SGLT)-1-mediated glucose uptake modulates enterocytic cell death induced by bacterial lipopolysaccharide. The aim of this study was to examine whether enhanced epithelial SGLT-1 activity may constitute a novel mechanism of host defense against *G. duodenalis*-induced apoptosis. SGLT-1-transfected Caco-2 cells were exposed to *G. duodenalis* products in low (5 mM) or high (25 mM) glucose media. In low glucose environments, *G. duodenalis*-induced caspase-3 activation and DNA fragmentation in these cells. These apoptotic phenomena were abolished in the presence of high glucose. A soluble proteolytic fraction of *G. duodenalis* was found to upregulate SGLT-1-mediated glucose uptake in a dose- and time-dependent manner, in association with increased apical SGLT-1 expression on epithelial cells. Kinetic analysis showed that this phenomenon resulted from an increase in the maximal rate of sugar transport (V_{max}) by SGLT-1, with no change in the affinity constant (K_m). The addition of phloridzin (a competitive inhibitor for glucose binding to SGLT-1) abolished the anti-apoptotic effects exerted by high glucose. Together, the findings indicate that SGLT-1-dependent glucose uptake may represent a novel epithelial cell rescue mechanism against *G. duodenalis*-induced apoptosis.

Keywords: *Giardia duodenalis*; Enterocytes; Apoptosis; SGLT-1; Glucose; Cytoprotection

1. Introduction

The protozoan parasite, *Giardia duodenalis* (syn. *Giardia lamblia*, *Giardia intestinalis*) is one of the most frequently identified etiologic agents of waterborne intestinal disease worldwide (Slifko et al., 2000; Savioli et al., 2006). *Giardia* trophozoites mostly colonize the upper small intestine and adhere to the apical surface of the epithelium. Symptoms due to this infection are characterized by acute or chronic diarrhea, dehydration, abdominal cramping and weight loss. Studies in human patients, in experimental animal models, or in cell culture systems, have established that the infection causes small intestinal structural and functional abnormalities. These may include apoptosis induced epithelial barrier defects, microvillous shortening, disaccharidase deficiencies, hypersecretion of chloride, as well as malabsorption of electrolytes and water associated with infiltration of intraepithelial lymphocytes (Belosevic et al., 1989; Buret et al., 1992; Scott et al., 2000, 2002, 2004; Troeger et al., 2007; Panaro et al., 2007).

In the intestine, enterocytes connected by tight junctions serve as the first line of defense against the influx

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of harmful luminal contents such as microbial pathogens and cytotoxic products. Physiological extrusion of senescent apoptotic enterocytes does not compromise intestinal barrier function (Madara, 1990; Watson et al., 2005). In contrast, exposure to enteric pathogens such as *G. duodenalis*, *Escherichia coli*, *Salmonella enteritica* or *Helicobacter pylori* induces excessive enterocytic apoptosis, which may adversely affect epithelial tight junctional integrity (Jones et al., 2000; Le'Negrate et al., 2001; Chin et al., 2002; Paesold et al., 2002; Scott et al., 2002; Yu et al., 2005; Troeger et al., 2007; Panaro et al., 2007). High concentrations of bacterial lipopolysaccharide (LPS) may also increase epithelial apoptosis and intestinal permeability (Yu et al., 2005, 2006; Chin et al., 2006). While it has been established that *G. duodenalis*-induced intestinal permeability defects require caspase-3 activation and appear to be strain-dependent (Teoh et al., 2000; Chin et al., 2002), the mechanisms responsible for *G. duodenalis*-induced epithelial apoptosis remain incompletely understood.

Cytoprotective mechanisms evolved by host cells represent the cornerstone of homeostasis and cell survival upon exposure to exogenous pathological pro-apoptotic stimuli. Glucose-mediated cytoprotection has been documented in a number of cell types, including myocardiocytes, vascular smooth muscle cells, mast cells, T cells and, most recently, in enterocytes (Schaffer et al., 2000; Hall et al., 2001; Malhotra et al., 2001; Yu et al., 2005). The mechanisms regulating this response remain poorly understood. The main apical transporter for active glucose uptake in intestinal epithelial cells is the sodium-dependent glucose co-transporter (SGLT)-1 (Hediger et al., 1987). SGLT-1 unidirectionally mediates glucose absorption from the intestinal lumen into epithelial cells. The basolateral transporter GLUT-2 facilitates diffusive transport of intracellular glucose into the interstitium and towards the bloodstream (Kimmich and Randles, 1981). Moreover, SGLT-1 co-transports glucose and sodium, which drives passive water uptake. This characteristic has been used for the development of oral rehydration therapy (ORT) to promote electrolyte balance, and manage hypersecretory diarrheal disease (Kimmich and Randles, 1984). Recent observations have described a novel cell rescue mechanism against LPS-induced apoptosis via SGLT-1 activation and enhanced glucose uptake into enterocytes (Yu et al., 2005, 2006). Whether this SGLT-1-mediated phenomenon confers cytoprotection against *G. duodenalis*-induced cellular apoptosis has yet to be explored.

The present study: (i) tested the hypothesis that high external glucose concentrations may protect human intestinal epithelial cells against apoptotic death caused by *G. duodenalis*; (ii) assessed the role of SGLT-1 as the glucose transporter responsible for the anti-apoptotic effect; and (iii) investigated the mechanism of *G. duodenalis*-induced SGLT-1 activation in these cells.

2. Materials and methods

2.1. Cell culture model

Human colonic Caco-2 cells transfected with native intestinal SGLT-1 (Turner et al., 1996) were grown in DMEM (Life technologies, Inc., Gaithersburg, MD) that contained 25 mM of glucose as previously described (Yu et al., 2005, 2006). The media was supplemented with 10% FBS, 15 mM Hepes, 100 U/ml Penicillin, 0.1 mg/ml Streptomycin (Sigma, St. Louis, MO) and 0.25 mg/ml Geneticin (Life technologies, Inc.) (Turner et al., 1996). None of the polarized intestinal epithelial cell lines available today express detectable levels of transepithelial Na^+-dependent glucose transport, which has been attributed to a deficiency of surface expression of SGLT-1 (Turner et al., 1996; Kipp et al., 2003). In these transfected cells, the expression of SGLT-1 protein on the apical membrane is associated with functional Na^+-dependent uptake of glucose, and physiological transduction pathways in response to SGLT-1 activation (Turner et al., 1996; Shiue et al., 2005; Hu et al., 2006). These cells were seeded in 8-well chamber slides (4×10^5 cells/well; Lab-Tek, Nalge Nunc, Rochester, NY), 24-well plates (10^6 cells/well; Costar, Corning Inc., Corning, NY), 6-well plates (6×10^6 cells/well, Costar) and 12-well transwells, which contained 1 cm^2 semi-permeable filter membrane with 0.4 μm pores (10^6 cells/well, Costar) and grown to confluency for 1 week at 37 °C with 5% CO_2 and 96% humidity for experiments. In all studies, cells were used between passages 21 and 27.

2.2. Giardia products

Giardia duodenalis strain NF was obtained from an epidemic outbreak of human giardiasis in Newfoundland, Canada (Chin et al., 2002). *Giardia duodenalis* trophozoites were grown axenically at 37 °C in Diamond's TYI-S33 media supplemented with piperacillin (Piprecil; Wyeth-Ayerst Canada Inc., Montreal, Que., Canada) in 15 ml polystyrene centrifuge tubes (Falcon, Becton–Dickinson and Co., Franklin Lakes, NJ) and sub-cultured every 7 days to maintain the line. Recent studies have demonstrated that live trophozoites, parasite sonicates, and spent growth media of *G. duodenalis* induce cell apoptosis and barrier defects in Caco-2 and SCBN epithelial cell lines (Teoh et al., 2000; Chin et al., 2002). For the purpose of using a reproducible stimulus, and in order to circumvent the physical interference of whole live parasites with the sugar uptake assay, *G. duodenalis* sonicates were used as the challenge in the current study. To harvest live *G. duodenalis* trophozoites, media and dead parasites were removed by aspiration and the tubes were filled with sterile PBS. The live trophozoites were harvested at log phase (48 h) by cold shock on ice for 20 min followed by centrifugation at 500*g* for 10 min at 4 °C. The pellet was washed once with 4 °C PBS and the number of trophozoites was counted using a